THE USE OF PHARMACOKINETICS TO OPTIMISE DOSING AND REFORMULATION OF ANTIRETROVIRALS FOR TREATMENT AND PREVENTION.

THESIS SUBMITTED IN ACCORDANCE WITH THE REQUIREMENTS OF THE UNIVERSITY OF LIVERPOOL FOR THE DEGREE OF DOCTOR IN PHILOSOPHY BY

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1 GLOSSARY

3TC	lamivudine
Ag	antigen
AIDS	acquired immunodeficiency syndrome
AUC	area under the concentration/time curve
AZT	azidothymidine (see also ZDV)
BMI	body mass index
cART	combination antiretroviral therapy
CDC	US Centers for Disease Control
Cmax	maximum concentration after dosing
Ctrough	concentration at the end of dosing interval
CV	coefficient of variation
CVF	cervicovaginal fluid
CVL	cervicovaginal lavage
d4T	stavudine
DNA	deoxyribose nucleic acid
DRV	darunavir
ECG	electrocardiograph
EFV	efavirenz
ETR	etravirine
FTC	emtricitabine
GM	geometric mean
GMR	geometric mean ratio
HIV	human immunodeficiency virus
IM	intramuscular
LLQ	lower limit of quantification
LPV	lopinavir
MSM	men who have sex with men
NNIBP	non-nucleoside inhibitor binding pocket
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
PEP	post-exposure prophylaxis
PrEP	pre-exposure prophylaxis
PSC	Protocol Steering Committee
QC	quality control
RAL	raltegravir
RF	rectal fluid
RLU	relative luminometer units
RPV	rilpivirine

RPV-LA	long-acting rilpivirine
RT	reverse transcriptase
rtv	ritonavir
SDN	solid-drug nanoparticle
SSAT	St. Stephens AIDS Trust
TDF	tenofovir disoproxil fumarate
Tmax	time to maximum concentration after dosing
TZM-bl	cell type used in HIV-1 endpoint neutralisation assay. TZM-bl cell line is derived from a HeLa cell clone that was engineered to express CD4, CCR5 and CXCR4
VL	viral load
WHO	World Health Organisation
ZDV	zidovudine

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2 INTRODUCTION

2.1 HIV: NATURAL HISTORY OF THE DISEASE. EVOLUTION FROM DISCOVERY TO DATE

2.1.1 DISCOVERY OF DISEASE EPIDEMIC

This work addresses the development of pharmacological therapeutic agents to enable better treatment of people infected with the Human Immunodeficiency Virus (HIV). The specific challenge in both the prevention and treatment of this condition marks one of the great successes of the last fifty years, but yet remains an incomplete solution to the problem with many people still living with the manifestation of the infection, Acquired Immunodeficiency Syndrome (AIDS), or dying from its complications.

It is almost four decades since the attention of the public and the media was drawn to a seeming new disease syndrome with alarming lethality and the ability to spread amongst the young population. The emergence of HIV/AIDS to become a global pandemic represented a most significant social and biological challenge, a disorder of uncertain aetiology, but embedded in interpersonal behaviour. Once the existence of the condition and the uncertainty surrounding its nature was acknowledged it rapidly caught the attention of the public and media, clinicians and scientists. Even before the discovery of the causative agent, there was a high index

of suspicion that the clinical syndrome which came to be known as AIDS (Acquired Immunodeficiency Syndrome), was caused by a highly infectious agent. Classical epidemiology indicated the likely infectious nature, based upon its pattern of spread and concurrent reports of outbreaks in geographically divergent locations. While it remained possible that the underlying cause of the syndrome might be related to environmental exposure to chemical toxins, recreational drug use or radioactive material, there was high suspicion of the causative agent being a zoonotic virus, and therefore in principle being amenable to therapeutic intervention with a suitable pharmacological compound. (Gazzard 1988; Dalgleish et al. 1995)

The aggressive nature of the natural of natural history of HIV was vividly evident during the first two decades when uncontrolled it became prevalent amongst infected patient populations. The considerable challenge was of a disease with protean and unusual manifestations system, in which no organ or tissue was seemingly unaffected. The obvious cachectic loss of weight, together with severe constitutional symptoms could be related directly to a variety of causes of gastrointestinal disintegration. However, differentiating the secondary opportunistic infections and malignancies from the primary cause associated with a profoundly immune-depleted state required careful clinical observation and investigation. In time, these gave indication that any putative causative pathogen must display a negative lymphtropic action, wherein the very orchestrators of the immune system was disrupted. Thus, the defensive surveillance against invading pathogens depended were potential the target for destruction; CD4 cell-membrane

bearing T-lymphocytes and some macrophages. This would come to define the fundamental nature of the challenges faced in all efforts that have been taken to date to combat the disease. This demonstration of this fact represented a major step forward as it provided an accessible, and readily available early biomarker for the presence and progress of the disorder within the routine clinical context.. By indicating the extent of depletion of the circulating pool of helper T cells, it allowed both a presumptive diagnosis of the presence of this syndrome as well as a statement about the extent or severity to which the immune system had already been damaged. It allowed an immediate assessment and also provided a means through which the variable progression of the disorder could be followed over time

2.1.2 A Syndrome; Search for a Cause

The early epidemiology identified clusters of people at high risk of the disease, including men who have sex with men, heroin users, haemophilia patients and US immigrants from Haiti. A focus on these groups led to the identification of the causative agent as a slow-incubating *Lentivirus*, a genus of the family of *Retroviridae* in 1983. This was in the year after the US Centers for Disease Control (CDC) had named the syndrome AIDS, confirming that different appellations given to each observed cluster, were indications of a common disease process.

In common with all retroviruses, a *lentivirus* is a single-stranded positive sense RNA virus contained within an envelope, which in order to replicate by integration

of a nuclear DNA intermediate into host chromosomes, first requires transcription of the positive RNA strand into double-stranded DNA by a virally-encoded reverse transcriptase (RT) enzyme in the cytoplasm. This RT enzyme, which accompanies the viral genome within the infectious particle both reverse transcribes the complementary DNA (cDNA) strand from the viral RNA, before acting as a DNA-dependent DNA polymerase to transcribe the positive sense strand from this cDNA to form the double stranded pair. Simultaneously, its ribonuclease activity due to a domain RNAase H, degrades the RNA strand whilst the cDNA strand is being reverse transcribed. The lack of error-correction in this mechanism, underpins the continuous generation of all possible mutations in infected cells in a host, the vast majority of which result in defective "junk" gene transcripts which results in self-termination of the replication cycle. Where mutations occur, either singly or in combination, which are compatible with replication their proportionate "success" in efficiency of replication relative to un-mutated wild-type virus transcripts prevents their domination of the heterogenous pool of virions, without the selective pressure of drug therapy, capable of interrupting the cycle.

Trials in the early 1990s, illustrated the initial success of early nucleotide reverse transcriptase inhibitors, which proved to be transient as the selective pressure provided by just one therapeutic molecule, allowed viral escape with emergence of mutations which retained replicative capacity in the presence of that drug. Even though early studies were conducted prior to the general availability of biotechnologic assays to detect and quantify the presence of virus in clinical samples, this viral escape could was indirectly reflected in recrudescence of prior

AIDS-defining clinical syndromes and laboratory indication of loss of circulating lymphocyte subsets.

It is possible to target each step of viral replication, from its first extracellular interaction with host cell membrane glycoproteins, through cell-wall fusion and internalisation of the viral particle in an uncoating process, at cytosolic reverse transcription followed by intra-nuclear chromosomal integration utilising virally encoded enzymes, in the post-translational assembly of an immature virion, with protease cleavage and steps of budding from the cytosol into extracellular space and the subsequent maturations steps for the viral particle to acquire full infectivity.

The ability to use small molecules interrupt this step of reverse transcription has been of importance since the very first earliest efforts to attenuate viral replication; subsequently through their combination with other agents to achieve effective suppression of replication to the extent that circulating viraemia could not be detected in circulating plasma by standard clinically available assays.

Inhibition of the reverse transcription step has been accomplished by two mechanisms of action.

The first, by direct covalent binding to the active site of the RT enzyme, by synthetic nucleoside or nucleotide analogues resulting in highly effective termination of elongation of the transcribed DNA chain. As a class, these are

collectively termed NRTI, reverse transcriptase inhibitors which are either nucleoside or mono-phosphorylated nucleotide analogues. These require intracellular activation, through 3 or 2 phosphorylation steps to form the active moiety.

The second mechanism of RT inhibition involved the development of molecules with the ability to bind to a region of the RT protein, adjacent to the active site in a hydrophobic pocket in what is described as the palm region, which interacts allosterically with the catalytic site. This region is common to all drugs of this class, termed non-nucleoside reverse transcriptase inhibitors (NNRTI), which on binding cause a conformational change at the binding site to form a pocket (the NNIBP – non-nucleoside inhibitor binding pocket). Further, the adjacent structure of the RT enzyme subunit also undergoes a destabilising structural adjustment, which alters the shape and thus binding affinity of the catalytic site. This destabilisation of the enzyme on the nucleic acid chain template, from which it transcribes, alters its catalytic site affinity for nucleotide binding, thus attenuating its ability to transcribe from viral RNA and effectively. This synergistic activity between NNRTI and NRTI is the underlying reason why a combination of a dual NRTI backbone, with a NNRTI third agent has proven to be an effective and durable mainstay of combination antiretroviral therapy, despite all three agents targeting a single replicative step.

2.2 THE SCOPE OF THE CHALLENGE

Globally, the availability of such NNRTI based combinations have been an important enabling factor of the ambitious goals, set by those working in the field of HIV, to be achieved. These stakeholders, including policy makers, programmers, national governments and community-based organisations, operate within the framework of global HIV targets to systematically set a series of goals to combat the epidemic, which have driven a successful agenda for global health.

In 2003, the first step towards a goal of universal access to HIV/AIDS prevention and treatment was the *"3 by 5"* initiative. This was launched by UNAIDS and WHO with a global TARGET to provide three million people living with HIV/AIDS in low- and middle-income countries with antiretroviral treatment by the end of 2005. This established the consensus principle of treatment as a human right, accessible to all who needed it, regardless of their location in the world or their individual ability or that of their country's health system to afford the purchase of life-preserving medication.

Whilst the target of 3 million on therapy was missed by a wide margin, 1.3 million people were able to start therapy, which itself tripled the number of people being treated and is estimated to have prevented 250,000 to 350,000 AIDS-related deaths worldwide. Even with this relative success, the scale and relative distribution of the challenge of low coverage of treatment at the time is shown in Figure 2:A with vast swathes of low and middle income African and Eurasian countries having less than 10% coverage.

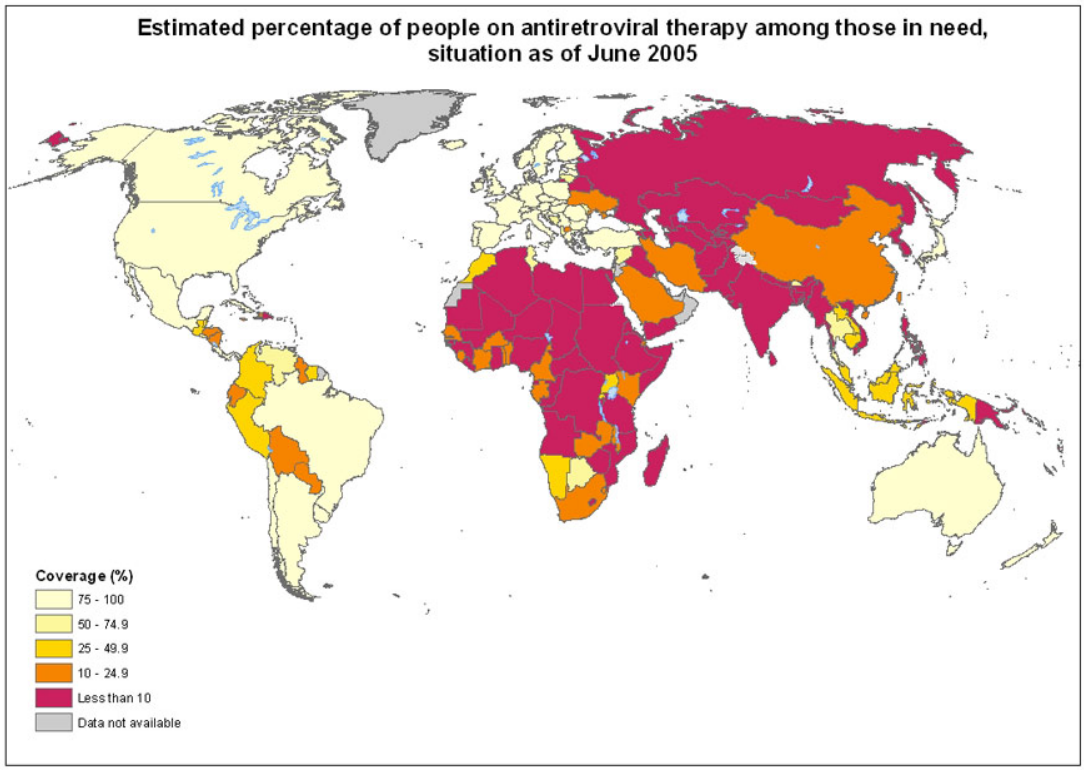

Figure 2:A WHO report on the global distribution of people living with HIV/AIDS on therapy in 2005. (http://www.who.int/hiv/facts/cov0605map/en/) accessed September 2017

In the decade which followed, global stakeholders continued to work together to develop successive statements of an agreed actions, with high-level signatories committing to coordinated efforts which acknowledged the iniquitous variability of the starting position, but resolved common goals across all territories (Table 2:A)

Table 2:A Progressive evolution of global framework for HIV/AIDS

2000	Millenium Development Goals target for 2015 included: • Goal 6: Combat HIV/AIDS, malaria and other diseases
2006	UN General Assembly High-Level Meeting: Political Declaration on HIV • review of the targets of the 2001 Declaration of Commitment on HIV/AIDS • countries signed the declaration to pledge their commitment to tackling the global HIV epidemic
2011	UN General Assembly: 60/262. Political Declaration on HIV/AIDS • reaffirmed the commitment to 2006 declaration • Set out the '15 by 15' target for 15 million people to be reached with HIV treatment by 2015
2011	UNAIDS developed ten interlinking targets in their 'Getting to Zero' strategy 2011-2015. • zero new HIV infections • zero AIDS-related deaths • zero discrimination.
2014	UNAIDS Fast track strategy; "90-90-90" 90 % targets for each step of: • testing and linkage to care • initiation of therapy • effective therapy resulting in viral suppression

Progress against these stated goals has been impressive within a relatively short timescale.

In 2015, of an estimated worldwide HIV prevalence of 36.7million the “15 by 15” treatment target was reached nine months before the end of the year, with 17 million on treatment by the end of 2015.

However, beyond these successes, the ongoing magnitude of burden of disease which saw an annual incidence of 2.1 million new HIV infections and 1.1 million AIDS-related deaths, remained stark and sobering. In July of 2011, published evidence from the HPTN053 confirmed the effectiveness of Treatment as Prevention (TasP) in preventing onward sexual transmission between partners of different serologic status. (Cohen et al. 2011) This evidence shows that both HIV incidence and AIDS deaths reported in 2015 are unnecessarily high statistics which would be sensitive to a further successes in the efforts to reduce the treatment gap between those infected those on treatment.

Thus, the public health challenge in 2017 has shifted from the situation of equipoise which existed in the early days of antiretroviral therapies which yielded intolerable and sometimes life-limiting toxicities, which meant that healthcare policies could cautiously to consider treatment.

Current evidence demonstrates that the balance, between the benefit of effective viral suppression against any potential deleterious effects due to that therapy, irrefutably supports immediate and ongoing treatment with suppressive antiretroviral therapy. (Strategies for Management of Antiretroviral Therapy SMART Study Group et al. 2006; Lodi et al. 2016) The current pharmacopeia provides varied

options to achieve this, with highly effective, well tolerated, simple therapies which are resilient and tolerating of suboptimal dosing adherence.

Within the context of this global situation, as stated, the work in this manuscript documents the exploration of three aspects of the existing topical challenges related to HIV treatment and prevention. The tools and techniques available within the study of clinical pharmacology are employed to address pragmatic clinical problems which are common to a wide spectrum of healthcare delivery scenarios where people living with HIV are managed.

2.3 Long-Acting Injectable HIV Prevention: Pharmacokinetic proof-of-concept

As described above, despite the advances in diagnosis and treatment of HIV infection, on a daily basis, new infections continue to occur in countries regardless of their wealth and status. In the US and in Europe the licensure of tenofovir/emtricitabine for HIV prevention provides a new and effective option for HIV prevention. In spite of this development, it is clear that daily oral prophylaxis ins not suited for all populations in whom the risk of HIV acquisition may be elevated. An example of this is the VOICE study, conducted in Sub-Saharan Africa, in which over five thousand women were randomised to receive oral or topical tenofovir as

PrEP. In this study, neither of the products were demonstrated to be effective. It became clear, through measurement of tenofovir in the plasma samples of participants that it was detectable in fewer than a third of them, indicating low levels of adherence. (Marrazzo et al. 2015) This effect was compounded by the fact that those participants at highest risk of infection were also found to be at highest risk of non-adherence to the products; women who were young and unmarried. However, this demographic group is routinely adherent to intermittent injectable progestins such as depot-medroxyprogesterone acetate, administered as a long-acting reversible contraceptive. (Morrison et al. 2015) Qualitative surveys on the attitudes and beliefs amongst populations at high risk of HIV infection indicate a potential interest in a long-acting injectable, including other risk groups such as men who have sex with men. (Eisingerich et al. 2012; Meyers et al. 2014)

Janssen Infectious Diseases BVBA, Belgium, the developers of the licensed oral formulation of rilpivirine are in the process of conducting a development programme of a nano-formulated suspension of rilpivirine as a long-acting therapeutic antiretroviral. At the early stages of its development, Janssen formed a collaborative agreement with the Bill and Melinda Gates Foundation (BMGF), in which they transferred the rights to develop this formulation as an HIV pre-exposure prophylactic to the Gates Foundation. In accordance with this agreement they would have a supportive role, providing technical expertise and a transfer of data and technology, but would not retain any rights to the commercialisation of long-acting rilpivirine for any future prophylactic indication.

Prior to the eventual study plan, named SSAT040, the initial planned proposal to study TMC-278 LA was a multi-centre study, with a single arm design which was planned to be conducted at 4 clinical research sites in the United Kingdom; 3 sites in London {St. Stephen's AIDS Trust as study sponsor, Imperial College NHS Trust and St. Thomas' Hospital} as well as the Royal Sussex County Hospital in Brighton. This initial study, given the code SSAT037, was developed as concept in 2009, receiving regulatory and ethics approvals at the start of 2010. The study - registered ClinicalTrials.gov Identifier NCT01049932, EudraCT: 2009-017631-17 – was designed to explore the primary endpoint of safety of administration of intramuscular 600mg TMC-278LA, after single dose with a second loading dose at 2 weeks and follow-up to presumed steady state following monthly doses. The aim was to accumulate data on 100 enrolled and evaluable subjects, with approximately 50 of African ancestry, and 50 females. This would have provided 50-subject-years of safety data in order to support and inform the design of a later large phase III global efficacy study.

As mentioned earlier, the parallel development paths of TMC-278LA for both HIV treatment (Tibotec) and HIV prevention, in this first exploratory pilot study were conducted simultaneously. In the first quarter of 2010, results from the ongoing small pharmacokinetic study TMC278-TiDP15-C150 ClinicalTrials.gov Identifier: NCT00741741 conducted by Tibotec Pharmaceuticals, indicated that the plasma pharmacokinetic exposure obtained with a single 600mg dose, afforded plasma drug exposure profiles which though in the desired therapeutic range, were lower than expected based on preclinical modelling simulations.

After extensive discussion with the SSAT037 study steering group stakeholders, the decision was made to terminate the study at a point after regulatory and ethical approvals had been obtained, and volunteers had been screened for participation but prior to any being enrolled.

Over the ensuing months in 2010, the study protocol was reviewed and abandoned to be replaced by a new study design, SSAT040 which was to be an adaptive design, dose-ranging exploratory PK study in healthy volunteers.

SSAT040 was the protocol designed to provide the first-in-man investigation of the long-acting rilpivirine, defining its plasma pharmacokinetics at a range of doses, whilst simultaneously exploring the compartmental pharmacokinetic exposures at tissue sites relevant to the sexual acquisition of HIV; vagina, cervix and rectum. Chapters 3 and 4 detail the results and conduct of this study.

2.4 INITIATION OF DUAL THERAPY IN ANTIRETROVIRAL NAÏVE PATIENTS?

Chapter 5 explores the potential utility of a conceptual approach to rationalisation of therapy which challenges the prevailing body of evidence and guidelines; is it feasible to use a two-drug combination of potent current-generation therapies, normally used as the third agent alongside a nucleos(t)ide backbone, to safely achieve viral suppression in HIV patients initiating treatment for the first time. At the time of conception of this study ClinicalTrials.gov Identifier: NCT01736761 in late 2012, the body of available evidence on this strategy was relatively limited and

the concept was unproven; Table 2 summarises the current published evidence on two-drug regimens used in initiation of antiretroviral therapies.

Specifically, the SSAT049 study, was an investigator-led study conducted under the regulatory sponsorship of the St. Stephen's AIDS Trust, in which the once-daily oral combination of ritonavir-boosted darunavir and rilpivirine was studied in an adaptive two-stage design. Funding for the study was provided by a grant from the manufacturer, Janssen. The study aimed to carefully investigate this combination in a controlled manner by utilising both sparse and intensive pharmacokinetics in the first month of therapy, combined with real-time assessment of pharmacodynamic outcome of viral decay to firstly assess the combination in patients whose circulating viraemic load was below 100,000 copies/mL in an initial cohort of ten patients. The guidance of a protocol steering committee, convened from experts with extensive experience of both clinical and pharmacokinetic, was utilised to provide monitoring oversight of the study and to make a "GO / NO GO" decision after the first ten patients, to allow further enrolment of patients with viraemic burdens above the threshold.

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2 *Table 2 Studies which evaluated nucleotide reverse transcriptase inhibitor-sparing regimens for initiation of therapy in naive patients..*

Study name & design		Study size (N)	Dual therapy	Reference
Boosted protease inhibitor plus integrase strand-transfer inhibitor				
RADAR (open-label)		85	DRV/rtv/RAL Vs Control (DRV/rtv/TDF/FTC)	(Bedimo et al. 2014)
ACTG 5262 (open label)		112	DRV/rtv/RAL	(Taiwo et al. 2011)
NEAT001 (open-label)		805	DRV/rtv/RAL Vs DRV/rtv/TDF/FTC	{LambertNiclot:2016dl} {Bernardino:2015el}
SPARTAN (open-label)	Unboosted protease inhibitor plus integrase strand-transfer inhibitor	94	ATV +RAL BiD Vs ATV/rtv/TDF/FTC	(Kozal et al. 2012)
PROGRESS (open-label)		206	LPV/rtv + RAL BiD Vs LPV/rtv/TDF/FTC	{Reynes:2011iv}{Reynes:2013ca}
Boosted protease inhibitor plus non-nucleoside reverse transcriptase inhibitor				
ACTG5142 (open-label)		753	LPV/rtv BiD + EFV Vs EFV/3TC/ {d4T or TDF or ZDV)	{Riddler:2008fv}

Study name & design	Study size (N)	Dual therapy	Reference
		Or LPV/rtv/3TC/ {d4T or TDF or ZDV)	
Boosted protease inhibitor plus CCR5 co-receptor antagonist			
MODERN (Double Blind)	797	DRV/rtv/MVC Vs DRV/rtv/TDF/FTC	{Stellbrink:2016ee}
A4001078 (open-label)	121	ATV/rtv/MVC Vs ATV/rtv/TDF/FTC	{Mills:2013cs}
MIDAS (open-label)	64	MVC/DRV/rtv	{Taiwo:2013eh}
Protease inhibitor plus 3TC			
GARDEL (open-label)	373	LPV/rtv/3TC Vs LPV/rtv/2 NRTIs	{Cahn:2014hc}

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2.5 A NOVEL METHOD FOR PRODUCING SOLID-DOSE NANOPARTICLE FORMULATIONS; EXPLORATION OF THEIR POTENTIAL FOR DOSE REDUCTION OF LICENSED ORAL THERAPIES.

In SSAT040 (Chapter 3), the potential of a solid-dose nanoparticle formulation of rilpivirine was studied for its ability to provide extended pharmacokinetic coverage when administered as a single-intramuscular injection.

Solid dose nano-formulations may also offer enhanced characteristics when applied to orally administered drugs. The Chemistry and Clinical Pharmacology groups at the University of Liverpool have developed a novel method of manufacture of solid-dose nano-particle formulations which have been chemically optimised and characterised using in-vitro tissue models of the interaction between these cell based systems and these nano-particles. By integrating this in a whole body mathematical model, they have been able to make a completely pre-clinical prediction of the dose kinetics of the studied drugs as a starting point for clinical phase studies. Chapter 6 details the ongoing study SSAT055, which explores in HIV-negative healthy volunteers, the pharmacokinetic behaviour of nano-formulations of the licensed antiretrovirals efavirenz and lopinavir (±ritonavir), comparing to the standard oral formulations.

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3 SSAT040: LONG-ACTING RILPIVIRINE

The candidate, AGAJ, acted as Chief Investigator for this study and was responsible for designing the concept, writing the protocol and lead the submissions for ethical and regulatory approvals. AGAJ established the local methodology for collection of samples and wrote standardisation manual and developed template proforma to ensure consistent collection of study samples and data. AGAJ was responsible for the clinical care of all participants on study, including informed consent and obtaining clinical history and assessing physical examination and determining eligibility and was responsible for administration of investigational product and collection of invasive samples. After bioanalysis was performed at the University of Liverpool bioanalysis facility - under GLP conditions - AGAJ was involved in compilation of the final results, presented the findings as an oral at the 2012 Conference on Retroviruses and Opportunistic Infection and wrote the manuscript as first author, which was published in Clinical Pharmacology and Therapeutics in June 2014.

3.1 BACKGROUND

Human Immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) refers to a strategy involving the use of antiretroviral drugs to decrease the risk of HIV infection in uninfected individuals whose behaviour would combine with local HIV prevalence to place them at a high risk of infection. In 2012, the use of tenofovir disoproxil fumatate and emtricitabine (TDF/FTC) in combination was approved by the US Food and Drug Administration for use as PrEP, based on the results of the iPrEx (Grant et

al. 2010) (van der Straten et al. 2012) study and Partners PrEP, (Baeten et al. 2012) with the former showing a 44% reduction in the incidence of HIV transmission in men who have sex with men as compared with placebo treatment, when combined with a comprehensive package of prevention. Partners PrEP showed a 67–75% relative reduction in the incidence of HIV infection using TDF/FTC among heterosexual couples in sexual partnerships containing one seronegative partner.

Although there is conceptual proof of PrEP in these specific contexts, recent negative results of two studies in women, FEMPrEP (Van Damme et al. 2012) and VOICE, (Marrazzo et al. 2013) showed no evidence of benefit of daily oral TDF/FTC. These negative outcomes were later ascribed to suboptimal adherence to the dosing regimen, thus indicating the need for high motivation in order to attain prevention success. Therefore, durable adherence is critical for a successful long-term prevention strategy. (Grant et al. 2010; Baeten et al. 2012; Marrazzo et al. 2013) In addition, the potential for side effects and toxicities associated with the use of TDF/ FTC (Grant et al. 2010; Thigpen et al. 2012) remains a concern due to its widespread administration as HIV PrEP.

An optimal PrEP therapy should be safe to administer and be readily distributed to the relevant target tissues in concentrations that are sufficient to provide protection against HIV infection. Ideally, PrEP agents should be characterized by convenient dosing and by routes of administration that do not depend on the recipient maintaining daily adherence to dosing. The nonnucleoside reverse-transcriptase inhibitor RPV is a diarylpyrimidine derivative that was approved by the Food and Drug Administration in 2011 for oral administration for the treatment of

HIV infection in combination with other ARV drugs.(Azijn et al. 2010; Ford et al. 2011) A parenteral formulation of rilpivirine (RPV-LA) with prolonged pharmacokinetic (PK) exposure is being developed, enabling improved adherence to ARV treatment over prolonged periods and having potential as an agent for HIV PrEP.(Baert et al. 2009; Grant et al. 2010; van't Klooster et al. 2010) The potential advantages of a long-acting formulation include infrequent parenteral administration and a low potential for gastrointestinal side effects associated with lifelong oral ARV intake.

For HIV prevention, it is important that the traditional sequence of the drug development phases be followed as closely as possible to address whether a PrEP agent is safe and effective for use in humans. However, dose optimization studies are challenging because protective concentration targets in both plasma and the genital/rectal compartments are unknown. Therefore, they must be inferred from treatment efficacy studies, animal models, and/or ex vivo pharmacodynamic (PD) experiments. We performed an adaptive design study to determine the plasma PK of RPV-LA and to measure, for the first time, RPV concentrations in cervicovaginal fluid (CVF), rectal fluid (RF), and tissue from the female genital tract and male rectum, after i.m. administration of a range of doses to HIV-negative volunteers. The study also aimed to assess the safety and tolerability of i.m. injections at 300, 600, and 1,200 mg of RPV-LA and to determine the effect of RPV in genital fluid on HIV replication ex vivo.

3.2 METHODS

3.2.1 PROTOCOL DEVELOPMENT.

This study was a phase I, prospective, openlabel, exploratory dose-ranging study conducted at a single center (St. Stephen's Centre clinical trial unit, Chelsea and Westminster Hospital, London) with development of the protocol under the regulatory sponsorship of the St. Stephen's AIDS Trust. Funding for the study was provided by a grant from the Bill & Melinda Gates Foundation with the engagement of Tibotec (now Janssen R&D Infectious Diseases), which provided the investigational agent, provided protocol oversight, and formed part of the protocol steering committee. The protocol was approved by the Medicines Healthcare Regulatory Agency, UK, and ethical approval was obtained, before commencement of the study and after each protocol amendment, from the National Research Ethics Service, UK. The protocol concept was developed as an adaptive exploratory design with the aim of investigating RPV exposure in plasma, fluids, and tissues from the female genital tract and male rectum at up to four different doses of RPV-LA administered i.m.: 300 or 600 mg, with the option to explore either a 1,200 mg or a 150 mg dose, dependent on pharmacokinetic drug exposure attained with the former doses relative to historic data with the licensed oral therapeutic dose (Figure 3:A SSAT040; Protocol Steering Committee algorithm).

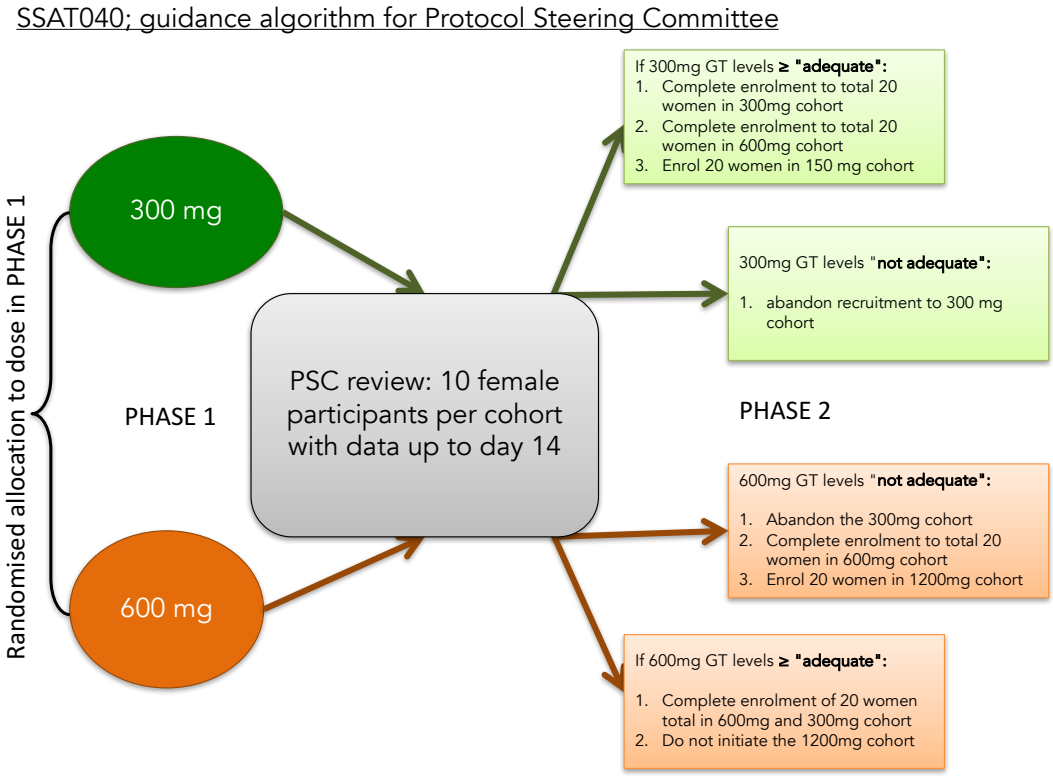


Figure 3:A SSAT040; Protocol Steering Committee algorithm

•The above figure was stated as guidance in the original protocol, approved by UK regulatory and ethics authorities, for the first Protocol Steering Committee (PSC) meeting.

•As approved, the protocol allowed the inclusion of 6 male volunteers, and 60 female volunteers total; in three dose groups of 20 each.

•PHASE 1 The first phase of study explored the plasma pharmacokinetics of 300mg (n=10) and 600mg (n=10), and the PSC was convened to compare the plasma exposure to day 28, comparing this for "adequacy" with a reference concentration from phase 3 treatment with oral rilpivirine of 50 ng/mL. Given that the data showed that 300mg was below this reference, with mean exposure for 600mg matching the reference range with no safety concerns the PSC deemed that the

second phase should proceed to enrol 10 volunteers to receive 1200mg and to complete enrolment to the 600mg dose.

•PHASE 2.

•The second PSC meeting reviewed plasma data from the phase 2 - 1200mg and 600mg cohorts - in addition to complete plasma exposure from the first phase in addition to genital compartment exposure in CV fluid. There were no further concerns regarding safety data with 600mg or 1200mg and plasma exposure at 600mg matching the reference at day 28 whilst 1200mg dose exceeded this concentration at days 28 and 56 following dose. The ratio of CV:plasma concentrations was greater than 1.0 at all timepoints. Viral inhibition studies on phases 1 & 2 were however unsuccessful, with directly aspirated vaginal fluid; the viscidity of the fluid causing technical failure of the assay.

•The decision of the second PSC therefore was to complete enrolment to the 1200mg dose, with an amendment to the collection method for CV fluid at days 28 and 56 using a lavage method rather than direct aspiration, in order to facilitate the viral inhibition assay.

•Given that there would be limited data available on viral inhibition at lower concentrations than those achieved with 1200mg, the decision was taken to also complete enrolment to the 300mg cohort with this amended collection method.

The study was conducted in three phases, with a review of data by the protocol steering committee after each of the first two phases to determine the subsequent protocol amendments. In the first phase, 6 male participants were recruited to receive a single dose of 600 mg and 10 female participants in each cohort received a single dose of 300 or 600 mg. After the first protocol steering committee review, the second phase enrolled two cohorts of 10 women to receive either a 600 or a 1,200 mg single dose, and the third phase completed the study with a further two cohorts of 10 women receiving either a 300 or a 1,200 mg single dose. In addition, on the basis of the protocol steering committee meetings, changes were made to the timing of vaginal tissue sampling for PK analysis (between phases) and to the timing and method of collection of CVF samples taken for exploratory PD assessments.

3.2.2 STUDY POPULATION AND RANDOMIZATION.

Male and non-pregnant, non-lactating female participants were eligible for enrolment if they provided written informed consent and met the eligibility criteria which were designed and reviewed by regulatory and research ethics authorities with the aim of recruiting healthy volunteers between the ages of 18 to 50 years who were HIV negative at the screening visit and whose and whose behavioural practices pertaining to risk, were likely to place them in the lowest category for HIV acquisition, both in the 6 months prior to enrolment and for at least the duration of study follow-up

Participants agreed to undergo regular HIV testing within the study and to abstain from sexual intercourse for the first 28 days of the trial and for 48 h before each subsequent study visit, before collection of genital or rectal samples.

The specific protocol defined eligibility criteria are detailed below.

3.2.2.1 *Inclusion Criteria*

Participants must satisfy all of the following criteria within 42 days prior to the baseline visit:

1. The ability to understand and sign a written informed consent form, prior to participation in any screening procedures and must be willing to comply with alltrial requirements.

2. Non-pregnant, non-lactating females (at least 40% will be of self-identified African ancestry)

3. Age between 18 to 50 years, inclusive.

4. Body Mass Index (BMI) of 16 to 35 kg/m2, inclusive.

5. Negative antibody/antigen combined test for HIV1 and HIV2.

6. Absence of any significant health problems (in the opinion of the investigator) on the basis of the screening procedures; including medical history, physical examination, vital signs, ECG.

7. Willing to undergo HIV testing, HIV discussion and receive HIV test results throughout the trial (according to the “UK National Guidelines for HIV Testing 2008”, www.bhiva.org).

8. Women of childbearing potential (WOCBP) must be using an adequate method of contraception (intrauterine device, condoms, anatomical sterility in self or partner) to avoid pregnancy throughout the trial and for a period of at least four months after the trial follow up visit (oral hormonal methods and implant contraceptives are allowed but only in combination with the additional protection of a barrier method). Males participating in sexual intercourse that could result in pregnancy must use condoms during the duration of the study and for up to four months following the follow up visit.

9. Willing to abstain from sexual intercourse (vaginal for females and receptive anal for males) for 48 hours prior to each trial visit (with complete abstinence in the first 28 days post-dose).

10. Females willing to refrain from the use of vaginal products or objects including, tampons, female condoms, cotton wool, rags, diaphragms, cervical caps (or any other vaginal barrier method), douches, lubricants, vibrators/dildos, and drying agents for 14 days prior to enrolment and for the duration of the trial. Males willing to refrain from the use of anal products or objects including douches, lubricants and vibrators/dildos for 14 days prior to enrolment and for the duration of the trial.

11. Likely to remain resident in the UK for the duration of the trial period.

12. Willing to consent to their personal details being entered onto The Over volunteering Prevention Scheme (TOPS) database.

13. Willing to provide photographic identification at each visit.

14. Registered with a GP in the UK

3.2.2.2 *Exclusion Criteria*

1. Any significant acute or chronic medical illness.

2. Evidence of organ dysfunction or any clinically significant deviation from normal in physical examination, vital signs, ECG or clinical laboratory determinations.

3. Positive blood screen for syphilis, hepatitis A (IgM) B (HBs Ag) and/or C antibodies.

4. Positive blood screen for HIV-1 and/or HIV-2 antibodies.

5. Positive screen for sexually transmitted infections at screening visit (if bacterial vaginosis or candidiasis detected at screen, these may be treated with test-of cure prior to enrolment).

6. Prolonged QT interval on screening ECG, or clinically significant change as judged by investigator.

7. High-risk behaviour for HIV infection which is defined as having one of the following within six months before trial day 0 (first dose):

i. had unprotected vaginal or anal sex with a known HIV infected person or a casual partner.

ii. engaged in sex work for money or drugs.

iii. acquired a sexually transmitted disease.

iv. having a high-risk partner either currently or in the previous six months

8. Clinically relevant alcohol or drug use (positive urine drug screen) or history of alcohol or drug use considered by the Investigator to be sufficient to hinder compliance with treatment, follow-up procedures or evaluation of adverse events.

9. Exposure to any investigational drug or placebo within 30 days of first dose of trial drug (additional check to be made on TOPS www.tops.org.uk).

10. History of severe drug allergy that in the opinion of the Investigator may increase the risk of developing an allergic reaction to the trial drug.

11. Use of any drug, including over-the-counter medications and herbal preparations, within two weeks prior to first dose of trial drug (unless approved or prescribed by the Investigator (for exceptions see section 5.2).

12. Females who are pregnant or breast-feeding.

13. Clinically significant laboratory abnormalities (according to normal range asdefined by central laboratory).

Six male participants were recruited, and all received a single 600 mg dose. Female participants were randomly assigned (within each study phase) to receive either of the two doses being studied. A statistician created randomization lists, stratified by phase, with blinded allocation maintained by means of sealed envelopes kept in a restricted-access pharmacy. Once allocation was performed, doses were administered on an open-label basis.

3.2.3 Study design

Eligible participants attended on the morning of day 0, when they received a single i.m. dose of RPV-LA between 8 and 10 am. Plasma (predose and 4- and 8 h postdose on day 0) and female genital or male RFs at 8 h postdose were collected for RPV PK analysis. Paired plasma and genital or RF samples were then collected on days 1, 3, 7, 11 (plasma only), 14, 21, 28, 42, 56, and 84 postdose. Paired tissue biopsies were taken from the pericervical vaginal fornix in women or the rectal mucosa in men at days 7 and 14, 14 and 28, or 28 and 56, depending on the study phase (Table 1).

3.2.4 Administered product

RPV-LA (formulation G001), available as a nanoparticle suspension with a concentration of 300 mg/ml, was given by i.m. injection into either buttock (300 mg—1 ml and 600 mg—2 ml) or both buttocks (1,200 mg—two 2 ml injections) on day 0.

3.2.5 Adverse Events

Adverse events were collected from the time of signing consent until each participant ended their involvement in the study. Adverse events were graded in accordance with the prevailing Division of AIDS Table for Grading of the severity of Adult and Paediatric Adverse Events (Version 1.0 December 2004 – clarification dated August 2009). Detail of the grading applied to events occurring at the injection site are shown in Table 3.

Table 3 Excerpted grading scale from version 1.0 of DAIDS grading scale for adverse events, with specific detail of adverse events related to injection site reactions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) or Tenderness (pain when area is touched)	Pain/ tenderness causing no or minimal limitation of use of limb	Pain/ tenderness limiting use of limb OR Pain/ tenderness causing greater than minimal interference with usual social and functional activities	Pain/ tenderness causing inability to perform usual social and functional activities	Pain/ tenderness causing inability to perform basic self-care function OR Hospitalisation (other than emergency room visit) indicated for management of pain/ tenderness
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm2 – 81cm2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)

3.2.6 SAMPLE COLLECTION

3.2.6.1 *Plasma*

At each scheduled time point, 6 ml whole blood was collected into lithium heparin Vacutainer vials (BD Biosciences, San Jose CA) and immediately placed on wet ice inside a light-protective container to prevent photo-degeneration before centrifugation at 1,000g for 10 min at 4 °C (within 2 h). Plasma aliquots were stored in light-protective polypropylene tubes at −20 °C until analysis.

3.2.6.2 *CVF*

Female participants collected samples by aspiration of vaginal secretions using a self-inserted disposable, sterile plastic volumetric device (Rovumeter, University of North Carolina School of Pharmacy)— a syringe-like device 135 mm long, with a constant outer diameter of 8 mm and a blunt, rounded distal end at which a 5-mm opening enables sample aspiration on applying suction to the plunger.

The undiluted aspirate was weighed, then chilled on wet ice in a light-protective polypropylene cryovial, before storage at −80 °C until analysis. During study phase III on days 0 (before dose), 28, and 56, CVL samples were collected, by aspiration after lavage of the cervix and vaginal vault with 10 ml of normal saline, in order to determine antiviral activity (PD analysis).

Therefore, at these study visits, direct aspiration of fluid was not performed in order to maximize the sample yield collected during the lavage process; instead, undiluted CVF was collected using Schirmer Tear-Test blotting paper strips (Intervet, Roseland, NJ) applied to the high vaginal mucosa with a vaginal speculum in place. The strips were weighed both before and after collection of the sample to enable

calculation of the volume of adsorbed fluid, and the strips were placed in a light-protective cryovial for storage at −80 °C until analysis.

3.2.6.3 *Rectal Fluid*

Samples for RF PK analysis were collected from male participants, after evacuating bowels, by adsorption onto Weck Cel cellulose spears (EYETEC; Network Medical Products, North Yorkshire, UK) that were placed in contact with apposed rectal mucosal surfaces (using a proctoscope) for at least 120 s; dry and wet weights were used to calculate the volume of fluid collected, and spears were placed inside light-protective cryovials for storage at −80 °C until analysis.

3.2.6.4 *Tissue*

Vaginal tissue was collected by biopsy; after insertion of the speculum, a 3 × 3 × 1 mm specimen was obtained by Sarratt biopsy forceps (Stericom, Chesham, UK), and samples were stored within 30 min in a light-protective cryovial at −80 °C until analysis. Rectal biopsies were obtained by the same methodology described above (following the insertion of a proctoscope) from a site in the rectum proximal to the dentate line to avoid highly innervated tissue.

The timing of

3.2.7 Analytical Methods

3.2.7.1 *Pharmacokinetics*

RPV concentrations in all matrices were quantified by validated high-pressure liquid chromatography–mass spectrometry using a Thermo triple quadrupole TSQ Ultra mass spectrometer (Thermo Electron Corporation, Hemel Hempstead, UK) operating in the positive ionization mode (selected reaction monitoring). (Azijn et al. 2010; Else et al. 2014)

In brief, a stable isotope–labeled internal standard (13C-d4-Rilpivirine; 20 µl, 80 ng/ml) was added to plasma, CVF, and tissue (VT and RT) samples (100 µl per sample), followed by extraction with protein precipitation (acetonitrile/water; 5:1 vol/vol) and was quantified using an RPV-spiked plasma calibration curve (0·5–400 ng/ml; 100 µl per calibrator level; in duplicate).

Due to its acidic (pH 4–5) and viscous nature, CVF was diluted 1:4 with a known volume of phosphate-buffered saline (1 mmol/l; adjusted to pH 4.5 with orthophosphoric acid) in order to create a homogeneous matrix and to improve pipetting accuracy. A 100-µl aliquot was then transferred to glass tubes, and the relevant dilution factor was recorded and these imputed values were entered into the analytical software.

For tissue biopsies, the weight of tissue (in milligrams) was recorded before extraction. Tissue biopsies were transferred to a MINILYS tissue homogenizer (Bertin Technologies, Bordeaux, France) and Precellys– Keramik kit (Bertin Technologies) containing 0·5-ml tubes prefilled with 14-mm ceramic beads, and made up to a volume of 100 µl with blank plasma. Inter- and intra-assay precision and accuracy for quality control (QC) samples at low (LQC), medium (MQC), and high (HQC) concentrations in plasma were <15%. The percentage recovery (internal standard normalized) of RPV from plasma (≥96%) was shown to be consistent, precise, and reproducible. Furthermore, the percentage recovery of RPV from direct CVF aspirates (~90%) and rectal tissue (RT) (~96%) using protein precipitation was equivalent to that of plasma, thus demonstrating that drug-free plasma serves as a suitable pseudo-matrix for quantification of RPV in these matrices.

RPV concentrations in RF were quantified using a plasma calibration curve spiked (50 µl; in duplicate) onto Weck Cel or PVA polyvinyl alcohol-based spears and extracted by liquid–liquid extraction (hexane/ ethyl acetate; 80:20 vol/vol). The calibration curve was linear over the 0.025–20 ng/sample. Inter- and intra-assay precision and accuracy for all QC concentrations were between 3 and 11%. The percentage recovery (internal standard normalized) of RPV absorbed onto Weck Cel or PVAbased spears, after liquid–liquid extraction, was ≥80%, and the effect of the sample matrix was minimal (<5% interference) when evaluated by spiking and postcolumn infusion experiments.

RPV concentrations in CVL were quantified using an RPV-spiked CVL calibration curve (100 µl per calibrator level) and extracted by protein precipitation

(acetonitrile/water; 5:1 vol/vol). RPV-free CVL (for spiking purposes) was obtained at baseline from the subjects undergoing CVL sampling and, subsequently, pooled. The CVL calibration curve was linear over 0.05–20 ng/ml.

RPV concentrations in all matrices were expressed as nanograms/milliliter. Tissue homogenate and rectal/vaginal fluid samples absorbed onto ophthalmic spears were quantified using a nanogram/sample calibration curve in order to account for variations in tissue weight and fluid volumes.

RPV concentrations in tissue (expressed as nanograms/milliliter) were calculated by converting x mg of tissue to a volume assuming a tissue density of 1.05 g/ml.

3.2.7.2 *Pharmacodynamics*

To determine the effect of RPV genital-tract concentrations on HIV replication in vitro, CVL samples were collected at baseline and on days 28 and 56 from women who received either 300 or 1,200 mg doses, during the third study phase. The antiviral activity of CVL samples was assessed against HIV-1BaL challenge of TZM-bl cells as previously described.7,10

In brief, TZM-bl cells were plated at 3 × 104/well and incubated overnight before exposure to approximately 103 TCID50 HIV-1BaL in the presence of undiluted CVL or control buffer (normal saline containing 200 µg/ ml bovine serum albumin) in triplicate wells. At 48 h postinfection, the inoculum was removed by washing once with 200 µl phosphate-buffered saline; cells were lysed in 100 µl luciferase cell culture lysis reagent (Promega, Madison, WI), and cell lysates were stored at −80 °C

until they were assessed for luciferase activity using a luciferase assay buffer (Promega).

3.2.8 Data Analysis

The calculated parameters for plasma and genital-tract RPV were maximum observed concentration (C_{max}), the area under the concentration–time curve from day 0 to 84 (AUC_{84d}), and the concentration measured at 84 days after the observed i.m. dose (C_{84d}).

All PK parameters were calculated using actual blood sampling times and non-compartmental modeling techniques (WinNonlin Phoenix (version 6·1; Pharsight, Mountain View, CA).

Dose proportionality was assessed by comparing dose-normalized (to 300 mg) and log-transformed PK data (AUC and C_{max}) using an analysis of variance and pairwise comparisons. In addition, a regression analysis of individual data based on the model

$y = \alpha.dose^{\beta}$ was applied, where y is either AUC or C_{max}, and β is the slope. A value of 1 for β indicates perfect dose proportionality.

Descriptive statistics, including GMs and 90% CIs, were calculated for all parameters.

Ratios of compartmental-to-systemic drug concentrations were calculated for each PK parameter (C_{max}, AUC84d, C84) and at all time points over the course of 84 days. The effects of gender, body weight, BMI, age, and ethnicity on systemic (plasma) and compartmental (female genital-tract) PK were evaluated using univariate and multivariate linear regression analyses. PK parameters were log transformed and dose normalized (to a dose of 300 mg). Variables were included in a full multivariate regression model if $P < 0.1$, and backward elimination ($P < 0.1$) was used to identify the most important predictors. Colinearity diagnostics were undertaken for expected interacting variables. All statistical analyses were performed using SPSS (version 20.0; IBM, New York, NY).

For the PK/PD correlation (CVL RPV concentration vs. HIV inhibition *in vitro*), a nonparametric Spearman's correlation was calculated using GraphPad Prism software (version. 6; GraphPad Prism, La Jolla, CA).

3.3 RESULTS

3.3.1 PARTICIPANT DEMOGRAPHICS, DISPOSITION AND SAFETY

Of the 89 women screened for this study, 60 were enrolled; in three phases of 20, they received a single dose of RPV-LA and provided blood, fluid, and tissue samples for analysis over the ensuing 84 days in each phase of the adaptive study design (Table 3:D Study Phases and sample schedule according to adaptive design modifications).

Table 3:D Study Phases and sample schedule according to adaptive design modifications

				Pharmacokinetic			Viral Inhibition
				Plasma samples	Cervical fluid Rectal fluid (male)	Tissue: Cervical Rectal (Male)	Cervico-vaginal fluid lavage
Study phase	Gender	Dose received (mg)	Number recruited	Sample schedule (study day)			
Phase 1	Male	600	6	Day 0 (pre-dose, 4 and 8 hour post dose) Days 1 to 84	Day 0 (8 hour post-dose) Days 1 to 84*	7 and 14	–
	Female	300	10			7 and 14 or 14 and 28	–
		600	10				–
Phase 2	Female	600	10			14 and 28	–
		1200	10				–
Phase 3	Female	300	10			28 and 56	0 (predose), 28 and 56
		1200	10				

* Days 1, 1, 3, 7, 11 (plasma only), 14, 21, 28, 42, 56 and 84

All female participants who received the single dose completed the study with no withdrawals due to adverse events. Six men were screened and found eligible to enrol, completing the study after receiving the 600 mg i.m. dose. Demographic information is presented in (Table 3:E Study Population Demographics and Dose Distribution); the three groups of women receiving 300, 600, and 1,200 mg doses had similar demographic characteristics.

Table 3:E Study Population Demographics and Dose Distribution

			Female		Male
		300 mg	600 mg	1200 mg	600 mg
Age (year)	Mean (± SD)	34 (±9)	35 (±8)	36 (±9)	36 (±15)
Height (m)	Median (range)	1·67 (1·51 – 1·79)	1·68 (1·58 – 1·76)	1·64 (1·52 – 1·76)	1·76 (1·69 – 1·81)
Weight (kg)		74·3 (51·2 – 96·7)	74·5 (55·6 – 100·4)	66·5 (47·8 – 90·6)	74·5 (61·0 – 100·4)
BMI ($kg.m^{-2}$)		26·6 (19·5 - 34·7)	26·6 (20·1 – 34·7)	24·7 (17·1 – 34·1)	24·5 (20·4 – 30·0)
Ethnicity					
Black		9 (45%)	14 (70%)	9 (45%)	1 (17%)
White		9 (45%)	5 (25%)	11 (55%)	5 (83%)
Asian		2 (10%)	1 (5%)	0	0

Participants tolerated the medication well. The majority of the adverse events experienced were mild in severity, and of those defined by the investigator as definitely or probably related to treatment, the most common were transient, self-limiting discomfort at the injection site and temporary presence of a palpable non-tender nodule. This was considered to be the deposit of the administered product;

no cases of this were complicated by any local signs of infection, and all cases resolved completely over the course of study involvement.

In the third phase of the study, one female participant receiving the 300 mg dose experienced a significant medical event of HIV infection after nonadherence to the use of barrier contraception with a new male sexual partner (subsequently found to be newly HIV seropositive) at approximately 6 weeks after receiving study medication. This will be discussed further in section 1 below.

3.3.2 Rilpivirine Plasma Pharmacokinetics

At the lowest dose of 300 mg, 19 of the 20 women had detectable plasma RPV above the lower limit of quantification (LLQ) at 4 h postadministration.

In women, the geometric mean (GM) and 90% confidence intervals (CIs) for RPV concentrations in blood plasma for all participants over 84 days after dosing are depicted in Figure 3:B,Figure 3:D,

Figure 3:F , and pharmacokinetic parameters are presented in Table 3:F.

Table 3:F RPV PK in female plasma and genital tract (CVF); (geometric mean, 90% confidence intervals)

	Plasma			CVF			$[RPV]_{CVF}/[RPV]_{PLASMA}$		
Dose	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg
N	20	20	20	20	20	20	20	20	20
PK parameter	GM 90%CI								
C_{max} (ng/mL)	34	82	160	67	99	200	2·0	1·2	1·3
	28, 40	69, 95	137, 183	42, 93	67, 132	155, 245	1·6, 2·5	0·8, 1·6	1·0, 1·5
T_{max} (days)	7·9	6·0	6·2	5·3	7·2	8·5	0·7	1·2	1·4
	4·3, 11·5	3·4, 8·6	4·3, 8·1	2·5, 8·2	3·1, 11·3	5·1, 11·9	0·1, 1·5	0·3, 2·1	0·1, 2·6
$t_{½}$ (days)	43	39	38	34	31	43	0·8	0·8	1·2
	28, 58	33, 45	30, 47	23, 45	25, 37	31, 56	0·6, 1·0	0·7, 1.0	0·6, 1·8

C_{max} = maximum concentration, T_{max} = time to reach C_{max}, $t_{½}$ = half-life, C_{28} = drug concentration 28 days post dose, C_{56} = drug concentration 56 days post dose, C_{84} = drug concentration 84 days post dose, AUC_{84d} = area under the curve from 0 to 84 days.

Table 3:C contd

	Plasma			CVF			$[RPV]_{CVF}/[RPV]_{PLASMA}$		
Dose	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg
C_{28}	19	44	83	25	39	85	1·2	0·9	1·0
(ng/mL)	16, 23	34, 55	67, 99	14, 36	18, 61	64, 106	0·8, 1·6	0·3, 1·5	0·8, 1·3
C_{56}	9	23	45	12	15	36	1·3	0·8	0·8
(ng/mL)	8, 11	19, 26	36, 55	7, 17	7, 22	26, 46	1·0, 1·7	0·5, 1·2	0·5, 1·1
C_{84}	6	16	30	10	12	36	1·7	0·9	1·2
(ng/mL)	6, 7	13, 19	24, 37	6, 15	5, 20	26, 46	1·1, 2·2	0·6, 1·3	0·7, 1·7
AUC_{84d}	1231	2934	5982	2027	3207	6500	1·7	1·1	1·1
(ng/day/mL)	1054,1408	2569,3300	5156,6807	1409,2645	2262,4152	5264,7735	1·3, 2.0	0·8, 1·4	0·9, 1·3

C_{max} = maximum concentration, T_{max} = time to reach C_{max}, $t_{½}$ = half-life, C_{28} = drug concentration 28 days post dose, C_{56} = drug concentration 56 days post dose, C_{84} = drug concentration 84 days post dose, AUC_{84d} = area under the curve from 0 to 84 days.

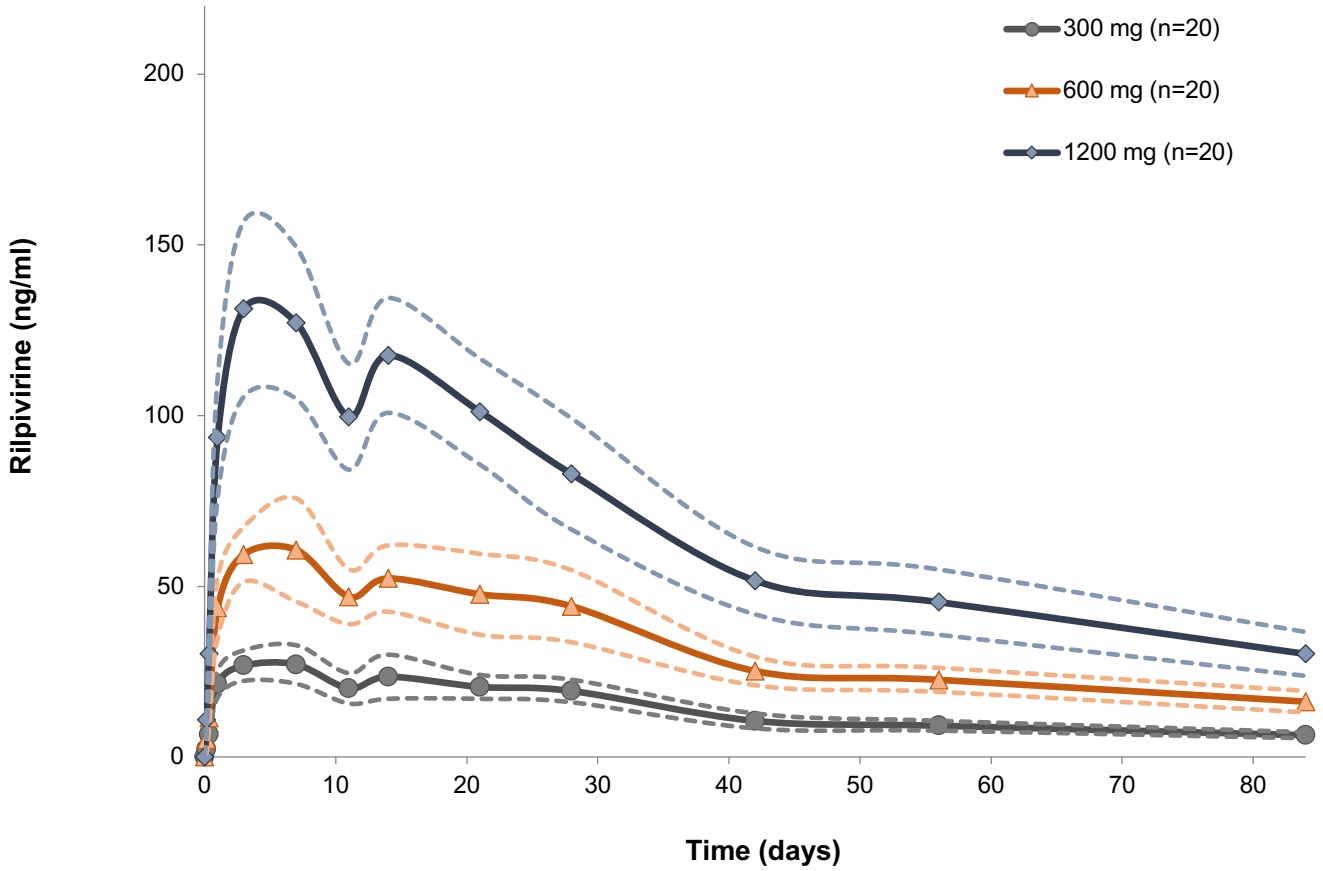


Figure 3:B Rilpivirine concentrations in women in plasma over 84 days post dose, when receiving LA-RPV i/m at 300, 600 or 1,200mg.

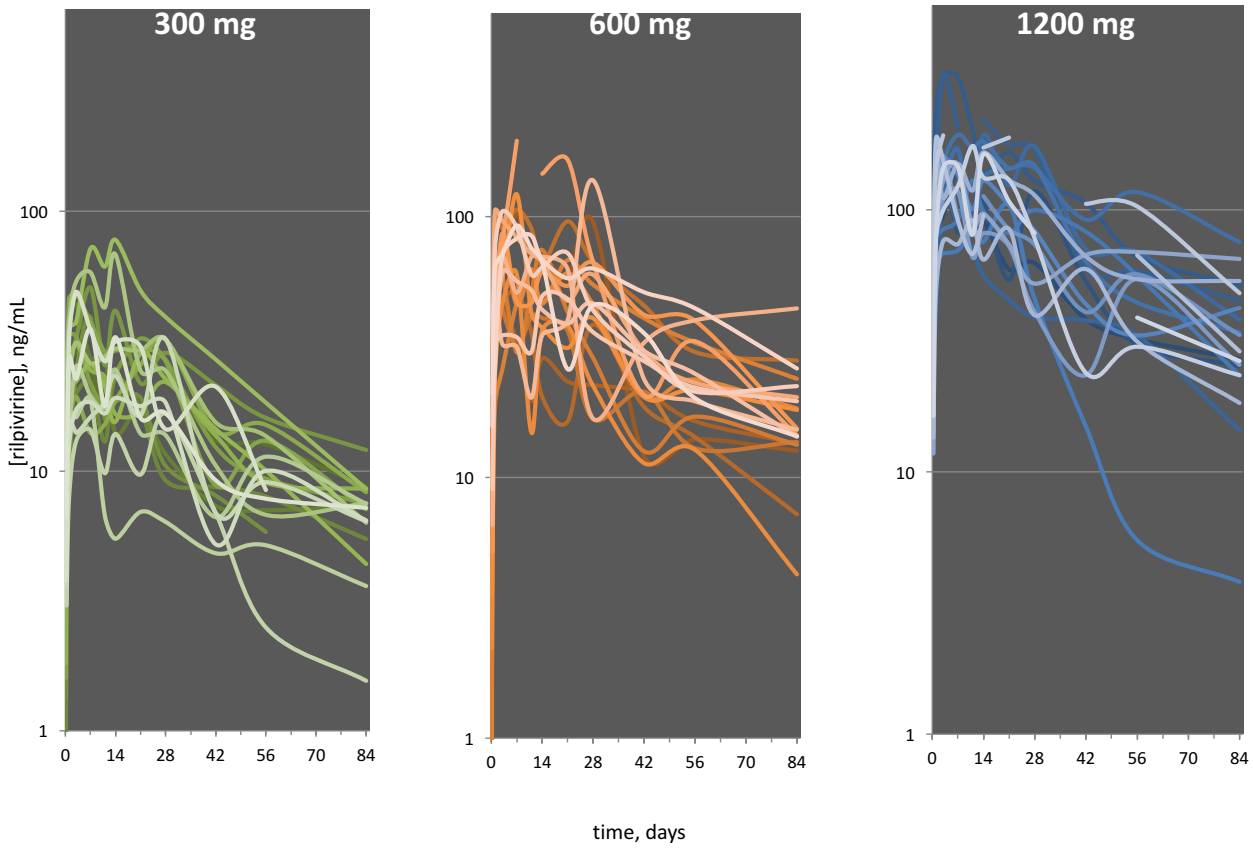
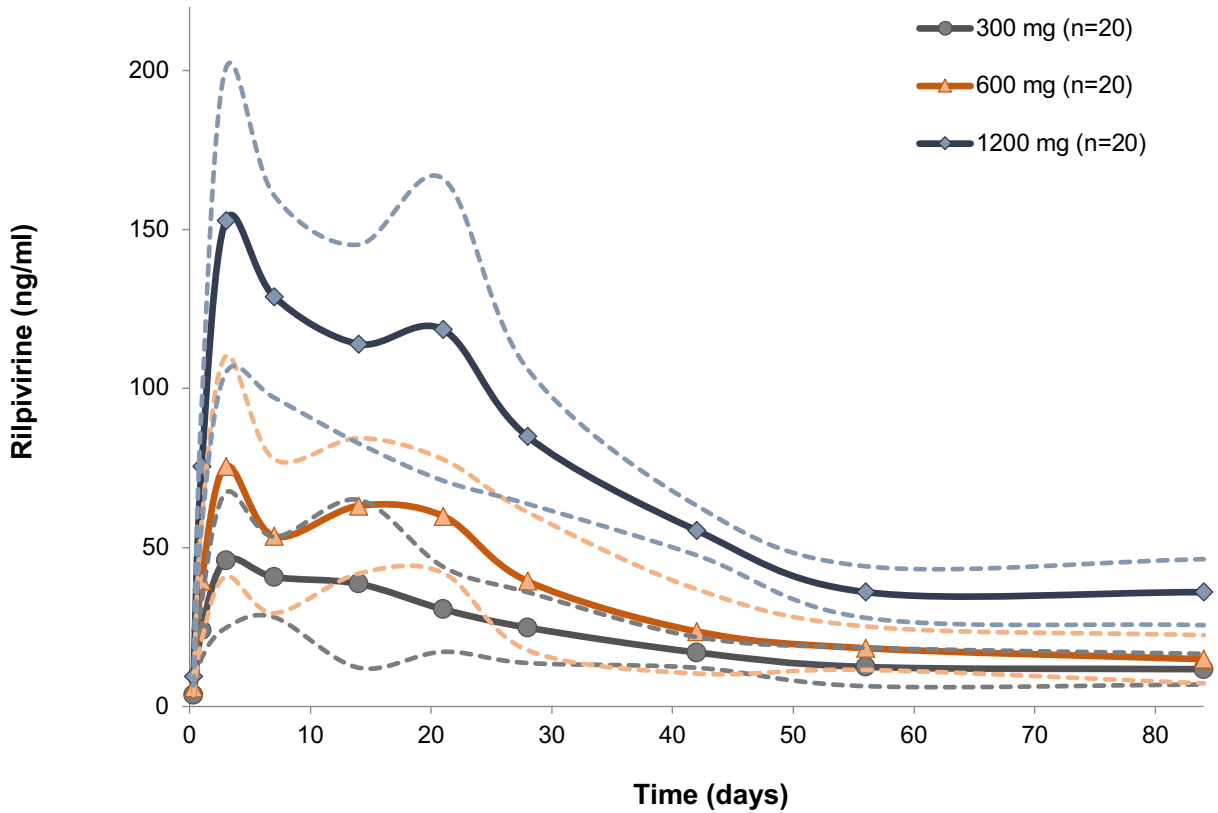


Figure 3:C Individual PLASMA rilpivirine concentration/time curves in female participants after receiving a single a) 300mg, b) 600mg or c) 1200mg dose.



1

2 Figure 3:D Rilpivirine concentrations in women in cercivo-vaginal fluid over 84 days post dose, when receiving LA-RPV i/m at 300, 600 or

3 1,200mg.

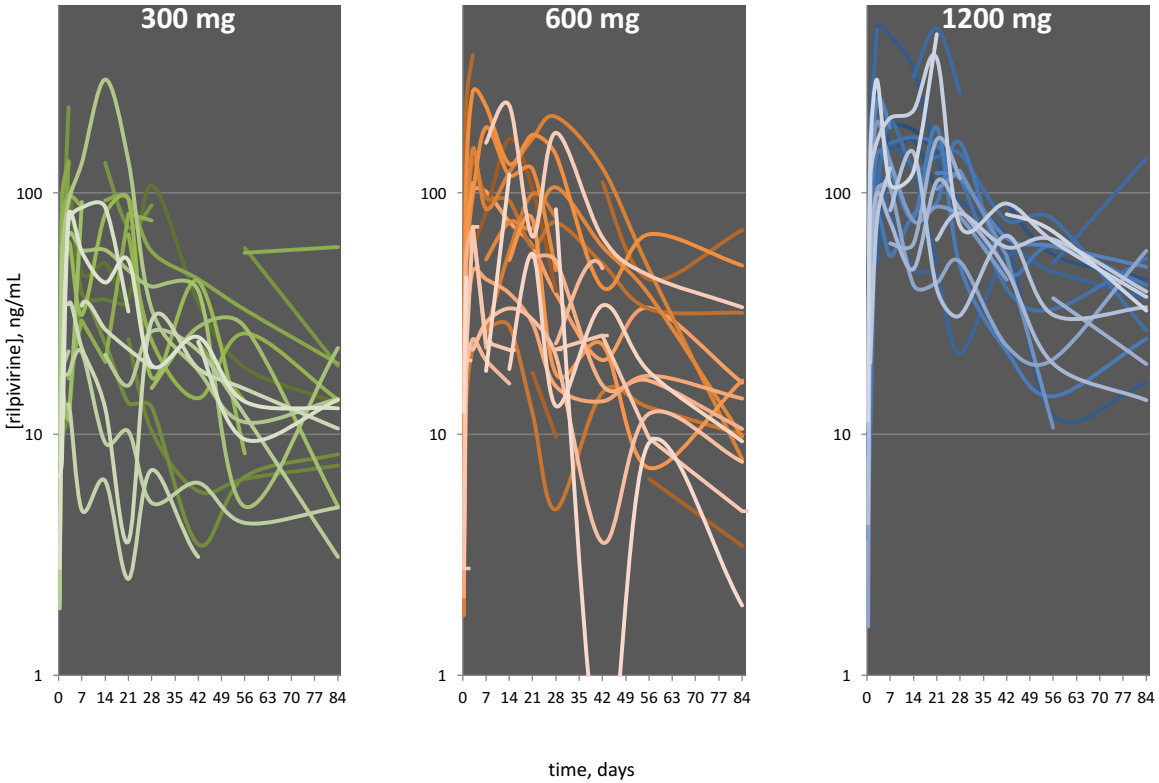
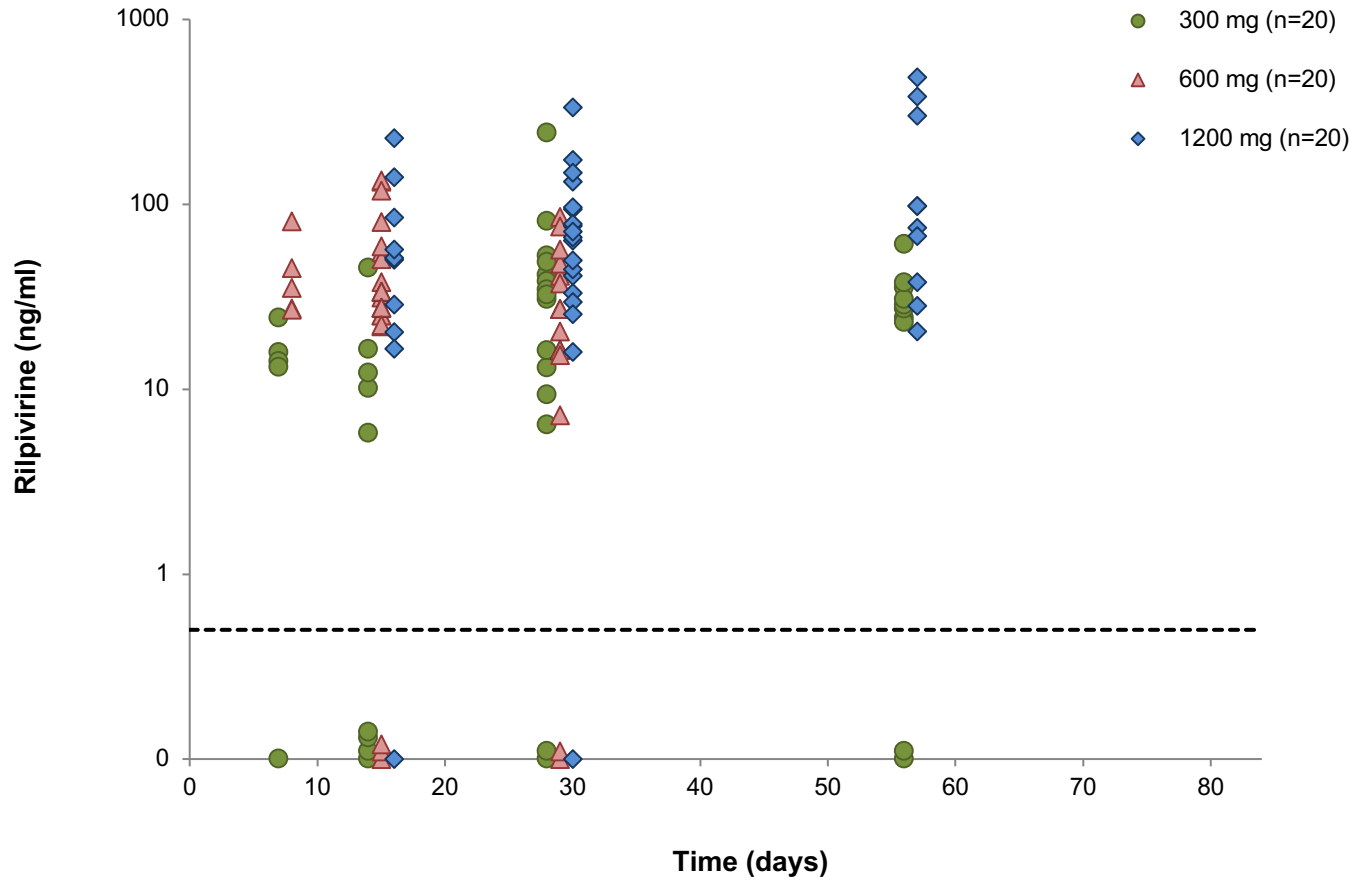


Figure 3:E Individual CERVICOVAGINAL rilpivirine concentration/time curves in participants after receiving a) 300mg, b) 600mg or c) 1200mg dose.



1

2 Figure 3:F Rilpivirine concentrations in women in vaginal tissue over 84 days post dose, when receiving LA-RPV i/m at 300, 600 or 1,200mg.

Mean peak concentration (C_{max}) in plasma of 33.7, 81.9, and 160.2 ng/ml was attained at 7.9, 6.0, and 6.2 days (mean time to C_{max} (T_{max})) after a single dose of 300, 600, and 1,200 mg, respectively. After this peak, the concentration–time curves describe a prolonged persistence of drug in plasma, with concentrations at 28 days (C_{28}) of 19.3, 44.2, and 82.9 ng/ml; at 56 days (C56) of 9.1, 22.6, and 45.4 ng/ml; and at 84 days (C_{84}) of 6.4, 16.2, and 30.2 ng/ml after the 300, 600, and 1,200 mg dose, respectively. Pairwise comparisons of plasma log area under the concentration–time curve (AUC) and C_{max} (dose normalized) did not result in any significant differences in plasma exposures among the three dosing groups ($P \geq 0.1$). In addition, the 90% CI of the regression slopes included unity. Dose proportionality was also demonstrated for rilpivirine CVF AUC and C_{max} (with the exception of C_{max} comparisons between the 1,200 and 300 mg doses; $P < 0.1$) using the same approach. These data suggest that both systemic and compartmental RPV concentrations are proportional to the administered i.m. dose.

In comparison, the six men receiving the 600 mg dose exhibited approximately 39% higher mean C_{max} as compared with women at the same dose (114 vs. 81.9 ng/ml), with subsequent higher concentrations at the sampling points C_{28} (33% higher) and C_{56} (21% higher) but with equivalent concentrations by day 84. This resulted in an increased overall exposure (AUC_{84d}) that was 32% above that measured in women at the same dose (Table 3:G RPV PK in male plasma and rectal compartment (RF); (geometric mean, 90% confidence interval).

Table 3:G RPV PK in male plasma and rectal compartment (RF); (geometric mean, 90% confidence interval)

	Plasma	RF	$[RPV]_{RF}/[RPV]_{PLASMA}$
Dose	600 mg	600 mg	600 mg
N	6	6	6
PK parameter	GM 90%CI		
C_{max} (ng/mL)	114·1	35·7	0·31
	88·8, 139·4	17·7, 53·6	0·20, 0·43
T_{max} (days)	5·0	6·2	1·24
	0, 11·7	0, 13·6	0, 3·94
$t_{½}$ (days)	30·5	17·8	0·61
	26·1, 35·0	9·3, 26·3	0·24, 0·97
C_{28} (ng/mL)	58·9	11·9	0·20
	42·3, 75·5	0, 35·0	0, 0·49
C_{56} (ng/mL)	27·4	5·9	0·22
	20·9, 33·8	3·4, 8·5	0·03, 0·41
C_{84} (ng/mL)	15·9	1·6	0·10
	13·9, 17·9	0, 3·4	0, 0·22
AUC_{84d} (ng/day/mL)	3873·4	935·0	0·24
	3283·7, 4463·2	350·2, 1519·8	0·10, 0·38

C_{max} = maximum concentration, T_{max} = time to reach C_{max}, $t_{½}$ = half-life, C_{28} = drug concentration 28 days post dose, C_{56} = drug concentration 56 days post dose, C_{84} = drug concentration 84 days post dose, AUC_{84d} = area under the curve from 0 to 84 days.

3.3.3 Cervico-Vaginal Fluid Pharmacokinetics

RPV was detectable above the assay LLQ in the first CVF sample, 8-h postdose, in all female participants in whom sample collection was successful (57/60; 95%), the exceptions being three volunteers receiving the 300 mg dose.

RPV CVF mean T_{max} mirrored that in plasma of between 5 and 8 days, attaining higher mean peak concentrations of 67.4, 99.3, and 199.9 ng/ml at 300, 600, and 1,200 mg, respectively (Table 3:F, Figure 3:D). Thereafter, RPV concentrations in CVF approximated those seen in plasma, with the GM $[RPV]_{CVF}/[RPV]_{PLASMA}$ ratio in paired samples maintained persistently at or above 0.8 at each dose level (Table 3:F). On day 84, RPV concentrations in CVF were still measurable, with a GM of 11.7, 14.9, and 36.0 ng/ml, respectively. Dose proportionality was also apparent in CVF, although inter-subject variation was higher than in the plasma compartment.

3.3.4 Rectal Fluid and Tissue concentrations

Rilpivirine was detectable in the first RF sample taken at 8 h postdose, in five of the six male participants, and in all six on day 1 (24 h postdose). The highest concentration in RF was observed at 6.2 days postdose and ranged from 15 to 92 ng/ml (Table 3:G, Figure 3:H), with the remaining measurable up to day 84 (from 0.4 to 7.4 ng/ml). RPV concentrations in RF were substantially lower than those in plasma or CVF. GM ratios of concentrations between RF and plasma ranged between 0.09 and 0.33 at different time points over the course of 84 days, with a ratio for the overall exposure, $AUC_{0–84d}$ RF/plasma of 0.24.

Rectal tissue RPV concentrations in men ranged from 67 to 128 ng/ml of tissue on day 7 and from 33 to 156 ng/ml of tissue on day 14 (Table 3:H, Figure 3:I), with tissue/plasma ratios from 0.7 to 1.2 on day 7, and from 0.5 to 1.3 on day 14.

Table 3:H RPV concentrations in VT and RT and their relation to plasma concentrations; (geometric mean, 90% confidence intervals)

Females	Plasma (n=20)			VT (n)			$[RPV]_{VT}/[RPV]_{PLASMA}$		
Dose	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg	300 mg	600 mg	1200 mg
PK parameter	GM 90%CI								
C_7 ng/mL	27·1 21·4, 32·7	60·6 45·5, 75·8	127·2 104·9, 149·4	16·4 14·5, 18·2 1<LLQ/(5)	39·4 31·2, 47·6 (5)	_	0·65 0·51, 0·80	0·72 0·40, 1·05	_
C_{14} ng/mL	23·4 17·0, 29·9	52·3 42·6, 62·0	117·7 100·8, 134·4	13·9 8·1, 19·7 4 <LLQ/(9)	41·4 29·1, 53·8 3<LLQ/(20)	53·9 28·6, 79·4 1<LLQ/(10)	0·59 0·30, 0·87	0·78 0·55, 1·01	0·45 0·22, 0·67
C_{28} ng/mL	19·3 16·0, 22·6	44·2 33·6, 54·7	82·9 66·6, 99·2	31·8 9·1, 54·5 2<LLQ/(15)	33·8 20·3, 47·3 3<LLQ/(15)	66·6 38·8, 94·4 1<LLQ (19)	1·70 0·58, 2·82	0·80 0·41, 1·19	0·83 0·48, 1·18
C_{56} ng/mL	9·1 7·7, 10·6	22·6 19·1, 26·1	45·4 35·8, 54·9	31·9 27·4, 36·4 2<LLQ/(10)	_	94·9 33·3, 156·6 (10)	3·79 2·35, 5·23	_	2·31 0·29, 4·90
Males	Plasma			RT			$[RPV]_{RT}/[RPV]_{PLASMA}$		
Dose	600 mg			600 mg			600 mg		
PK parameter	GM 90%CI (n)								
C_7 ng/mL	95·5 79·8, 111·1 (6)			93·7 75·6, 111·8 (6)			0·98 0·85, 1·11 (6)		
C_{14} ng/mL	78·2 63·7, 92·6 (6)			70·3 34·2, 106·3 (6)			0·90 0·59, 1·20 (6)		

C7, drug concentration 7 days postdose; C14, drug concentration 14 days postdose; C28, drug concentration 28 days postdose; C56, drug concentration 56 days postdose; LLQ, lower limit of quantification; RT, rectal tissue, VT, vaginal tissue.

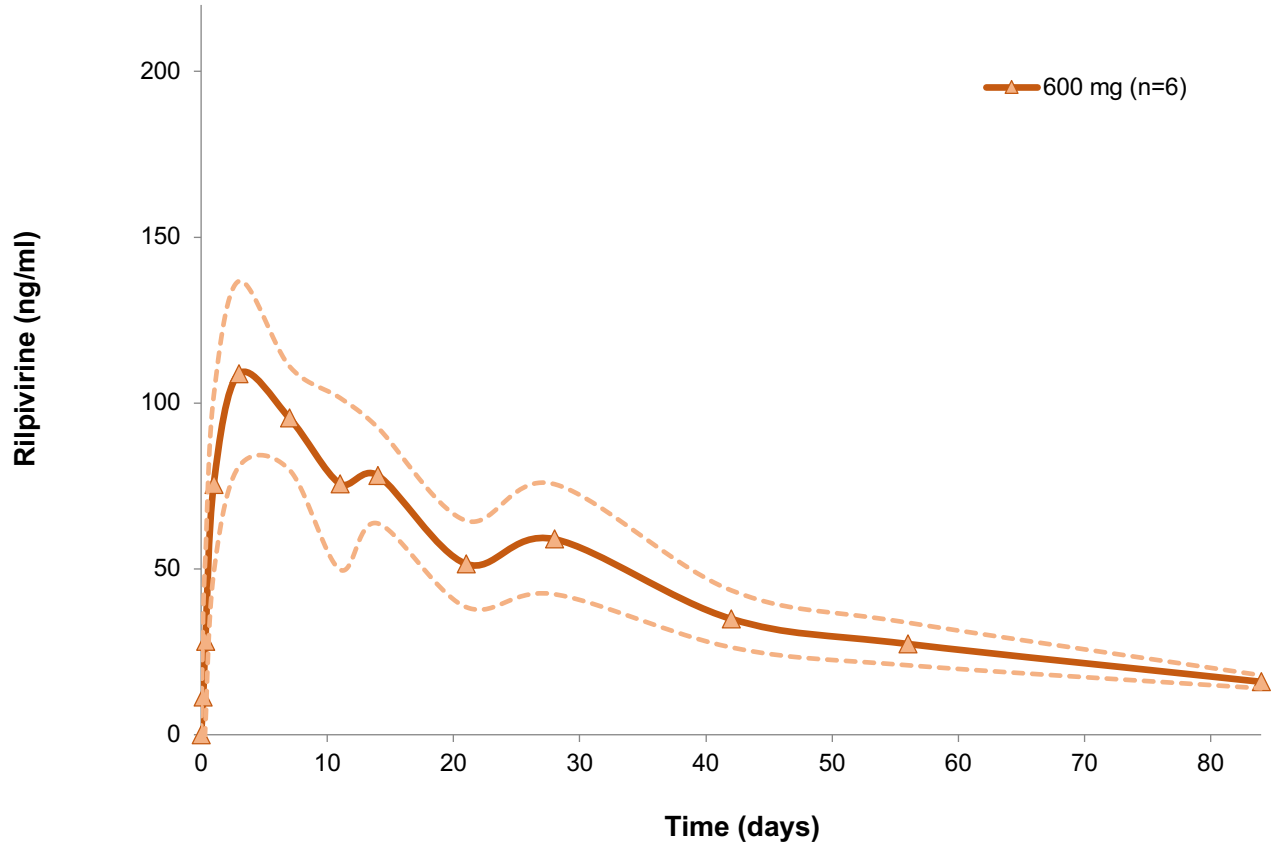


Figure 3:G Rilpivirine concentrations in men in plasma over 84 days postdose, when receiving long-acting RPV intramuscularly at 600mg

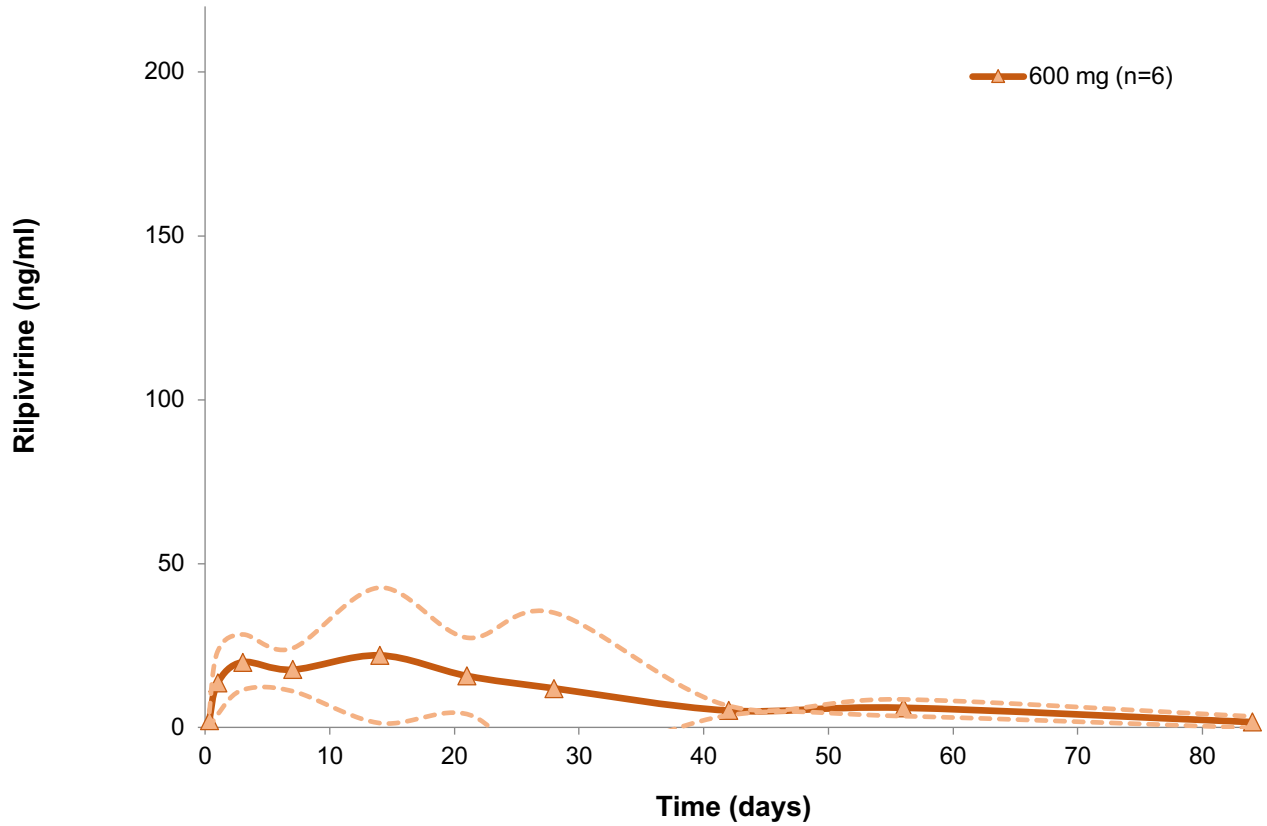


Figure 3:H Rilpivirine concentrations in men in rectal fluid over 84 days postdose, when receiving long-acting RPV intramuscularly at 600mg

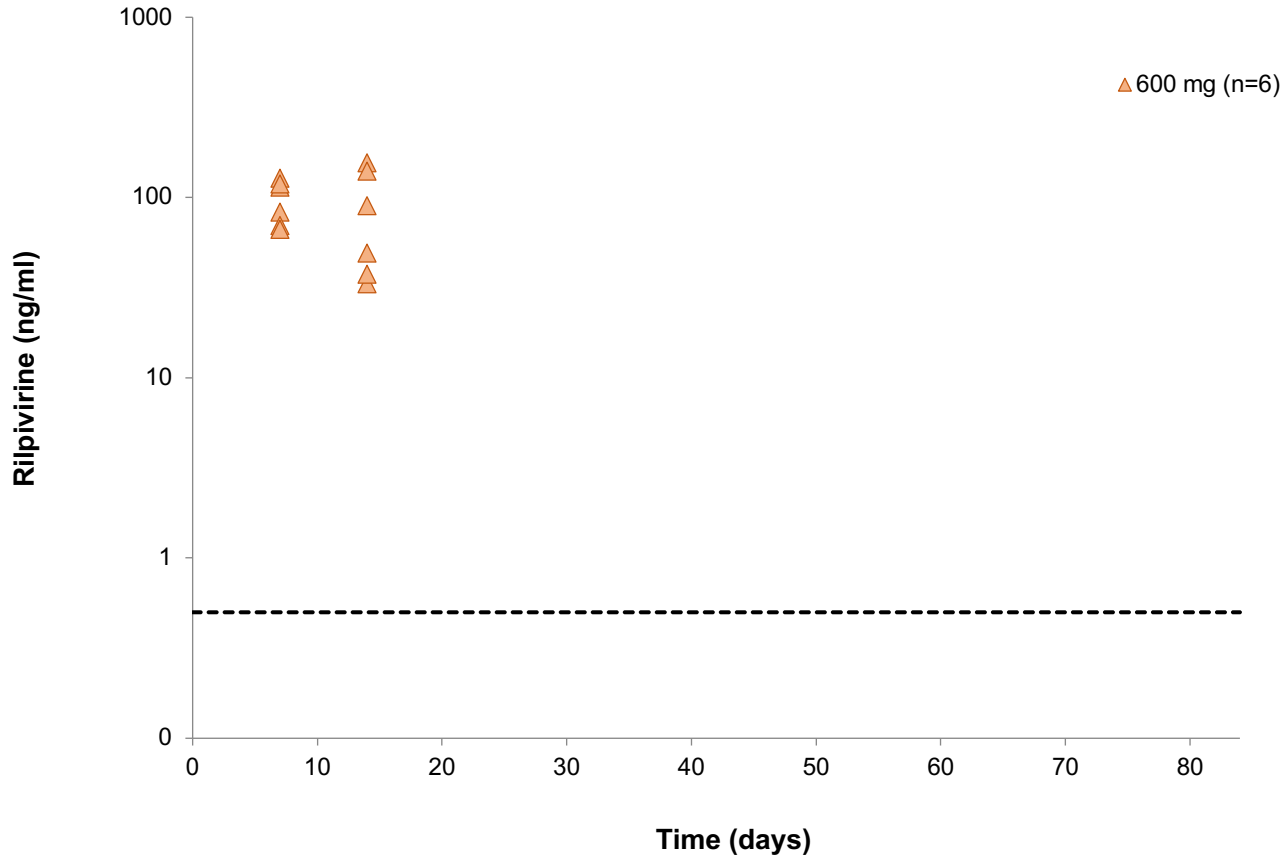
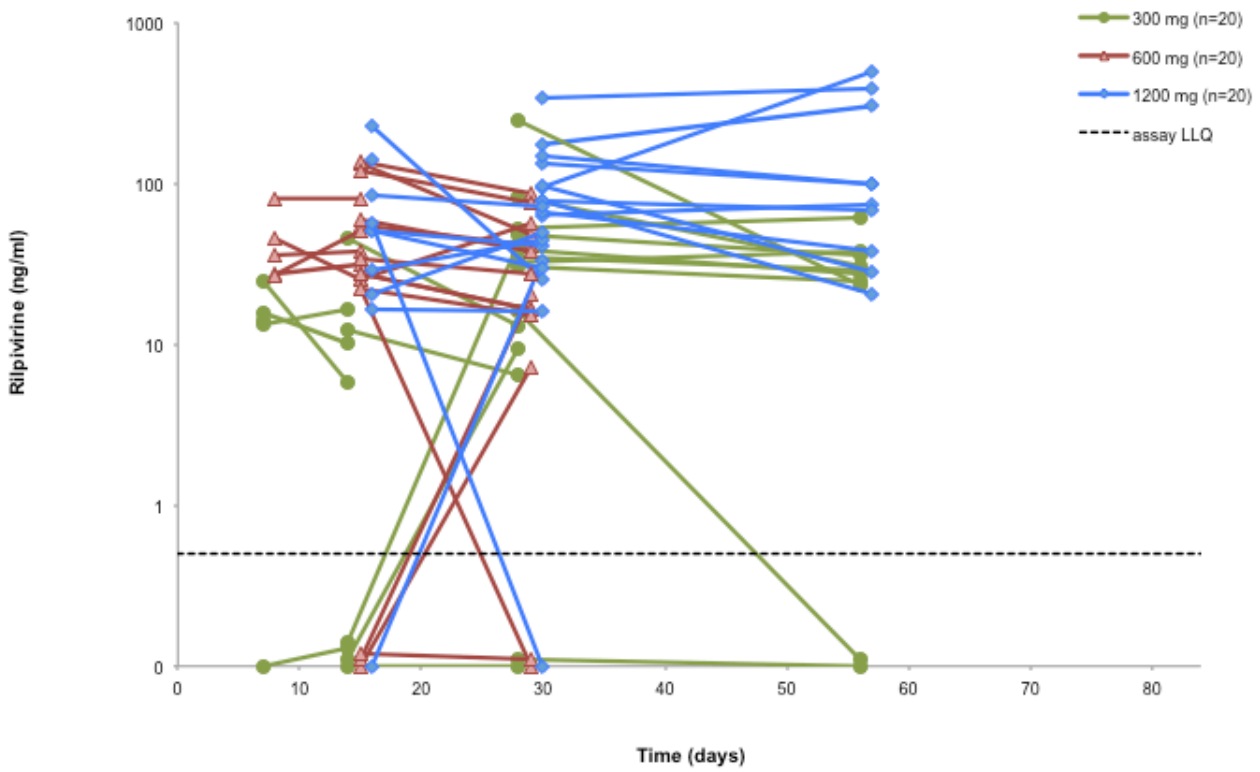


Figure 3:I Rilpivirine concentrations in men in rectal tissue over 84 days postdose, when receiving long-acting RPV intramuscularly at 600mg.



1

2 *Figure 3:J Paired rilpivirine tissue concentrations from individual participants over 84 days postdose after receiving RPV intramuscularly*
3 *at 300mg, 600mg and 1,200mg.*

4

3.3.5 Vaginal Tissue

Individual vaginal tissue (VT) RPV concentrations (GM, 90% CI) on days 7, 14, 28, and 56 are presented in

Figure 3:F and Figure 3:J, and concentrations with associated [RPV]VT/[RPV]PLASMA ratios are shown in Table 3:H.

In women in whom sampling was successful, RPV VT (ng/ml) concentrations (GM 90% CI presented in Table 3:H, in women receiving 300 mg ranged from 13 to 24 on day 7 (1 <LLQ), from 6 to 45 on day 14 (4 < LLQ), from 6 to 243 on day 28, (2 < LLQ), and from 23 to 61 ng/ml on day 56; with the GM of ratios in concentration—$[RPV]_{VT}/[RPV]_{PLASMA}$—on these days being 0.7, 0.6, 1.7, and 3.8, respectively.

In those receiving the 600 mg dose, concentrations ranged from 27 to 81, from 22 to 135 (3 < LLQ), and from 7 to 132 (3 <LLQ) in women who were on 600 mg on days 7 (n = 5), 14 (n =20), and 28 (n = 15). $RPV]_{VT}/[RPV]_{PLASMA}$ GM ratios were 0.7, 0.8, and 0.8 on days 7, 14, and 28, respectively.

At the highest dose (1,200 mg), concentrations ranged from 17 to 228 (1 < LLQ), from 16 to 335 (1 < LLQ), and from 21 to 487 in women on days 14 (n = 10), 28 (n = 19), and 56 (n = 10). $RPV]_{VT}/[RPV]_{PLASMA}$ GM ratios were 0.5, 0.8, and 2.3 on days 14, 28, and 56, respectively.

3.3.6 Covariate Analysis

A total of 520 paired plasma and CVF samples were available from the 60 female participants in the study. Plasma and CVF concentrations were significantly

correlated (r2 = 0.518; P <0.01). Furthermore, the association remained significant when stratifying by the dose administered (300 mg: r2 = 0.559; P <0.01; n = 173 pairs; 600 mg: r2 = 0.363; P < 0.01; n = 173 pairs; 1,200 mg: r2 = 0.449; P < 0.01; n = 174 pairs). Plasma and VT RPV concentrations from 100 paired samples were also significantly correlated (r2 = 0.139; P < 0.01; n = 100), but the association was somewhat weaker, and the significance was lost when stratifying by dose.

When investigating the effects of predictors on RPV plasma concentrations, in a multivariate model, female gender and body mass index (BMI) were found to be independently associated with RPV C_{max}. Female gender was associated with an approximately 30% decrease in RPV Cmax (P = 0.013), and a one-unit (kg/m^2) increase in BMI was associated with a 2.3% decrease in C_{max} (P = 0.028). In terms of the overall RPV exposure (AUC_{84d}) in plasma, only gender was a significant univariate predictor, in which women were associated with a ~28% decrease in RPV AUC_{84d}. Interestingly, there was no effect of either gender or BMI on RPV plasma concentrations beyond 28 days postdose. No effect of age, bodyweight, or ethnicity was observed for any of the RPV PK parameters in the plasma compartment.

In terms of predictors of RPV concentrations in the female genital tract (CVF and VT), in a multivariate analysis, age <40 years (P < 0.031), BMI > 25 kg/m^2 (P = 0.005), and RPV plasma concentrations (P = 0.005) were significant predictors of RPV concentrations in the female genital tract (CVF and VT), whereby age <40 years and BMI > 25 kg/m^2 were associated with a 35–40% reduction in RPV AUC and C_{max} in CVF, after adjusting for RPV plasma concentrations. There was no evidence of any co-linearity between age and BMI, and the effects of age and BMI were lost beyond

28 days postdose. In a univariate analysis, RPV CVF concentrations and BMI (>25 kg/m2) were predictors of RPV concentrations in VT at day 14. However, in a multivariate model, only BMI was a significant predictor, with an ~57% reduction in RPV tissue concentration ($P < 0.001$). No associations between BMI (or indeed other covariates) and RPV VT concentrations at days 28 or 56 were observed.

3.3.7 VIRAL INHIBITION IN CERVICOVAGINAL LAVAGE

RPV concentrations measured in cervicovaginal lavage (CVL) (GM; 90% CI) at days 28 and 56, respectively, were 0.45 ng/ml (0.08–0.83; 2 < LLQ) and 0.28 ng/ml (0.11–0.45; 4 < LLQ) for women who received the 300 mg dose, and 1.90 ng/ml (0.66–3.13) and 0.63 ng/ml (0–1.79) for those who were given the 1,200 mg dose. However, it should be recognised that the procedure for CVL collection resulted in a considerable dilution of the naturally occurring fluid. Despite this, RPV concentrations in CVL and paired "undiluted" tear-test strips, both of which were taken on days 28 and 56 in phase III participants, were significantly correlated (P < 0.00001; Spearman's r = 0.7). CVL collected from women receiving a single dose of RPV-LA 1,200 mg i.m. during the third study phase (n = 9) had significantly greater antiviral activity on both days 28 (93 ± 12%; mean ± SD) and 56 (78 ± 23%), as compared with the baseline activity (28 ± 64%) (Figure 3:K plot A). By contrast, CVL obtained from women who were on the lower i.m. dose of 300 mg did not result in a significant increase in antiviral activity at either time point, as compared with baseline (Figure 3:K plot B) and Figure 3:L). The activity correlated significantly (P < 0.0001; Spearman's r = 0.8) with RPV concentrations (Figure 3:K, plot C). Because the endogenous antiviral activity of CVL is highly variable,[10-12] the data were also analysed by subtracting the baseline inhibition from that observed at 28 and 56 days postdose, for each subject. The RPV-LA-driven inhibition after the 1,200 mg i.m. dose was higher at day 28 (71 ± 67%; mean ± SD) and persisted until day 56 (58 ± 74%; mean ± SD). By contrast, there was little to no increment after the 300 mg dose (Figure 3:M).

The volunteer who tested positive for HIV antibodies on study day 84 had detectable plasma HIV RNA of 370 and 175,060 copies/ml on study days 56 and 84, respectively. This volunteer received the lowest studied dose (300 mg i.m.) and the plasma and CVF RPV concentrations were 24.3 and 32.9 ng/ml, respectively, on day 28, 10.5 and 18.3 ng/ml, respectively, on day 42 (when presumed exposure to HIV occurred), 6.8 and 11.2 ng/ ml, respectively, on day 56, and 7.5 and 14.0 ng/ml, respectively, on day 84. There was minimal change in viral inhibition in CVL obtained from this subject on day 28 (66%) or on day 56 (55%) from that at baseline (49%). These findings indicate that the 300 mg dose is not sufficient to protect against HIV infection.

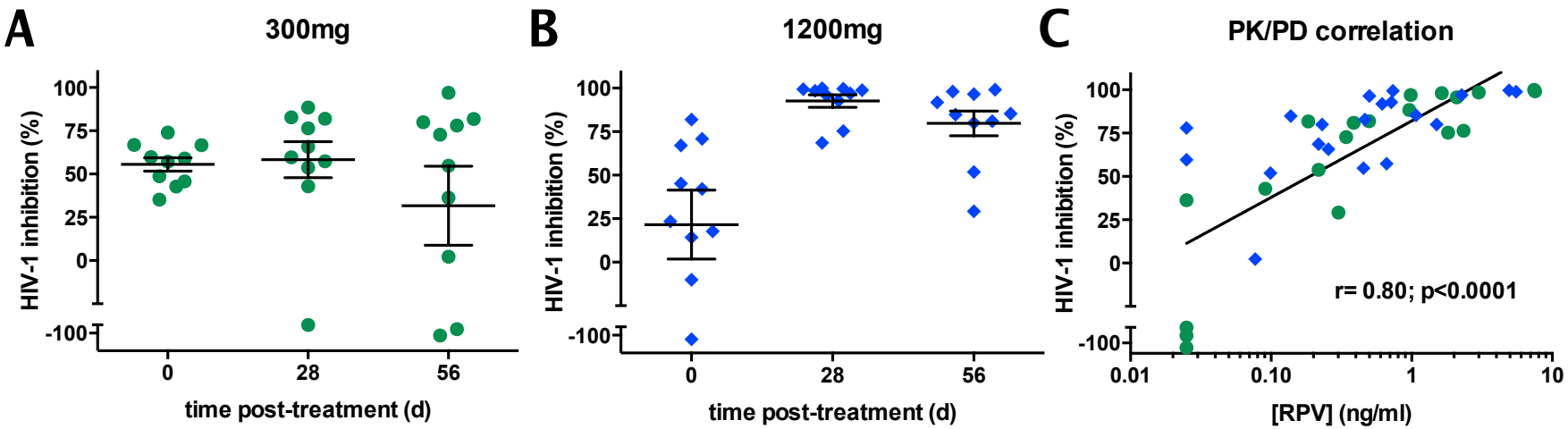


Figure 3:K HIV viral inhibition percentage measured ex vivo in cervicovaginal lavage in female participants at baseline and at 28 and 56 days postdose.

A 300mg, n = 10; **B** 1,200mg, n = 10; **C** Nonparametric Spearman's correlation between rilpivirine concentrations and viral inhibition at days 28 and 56.

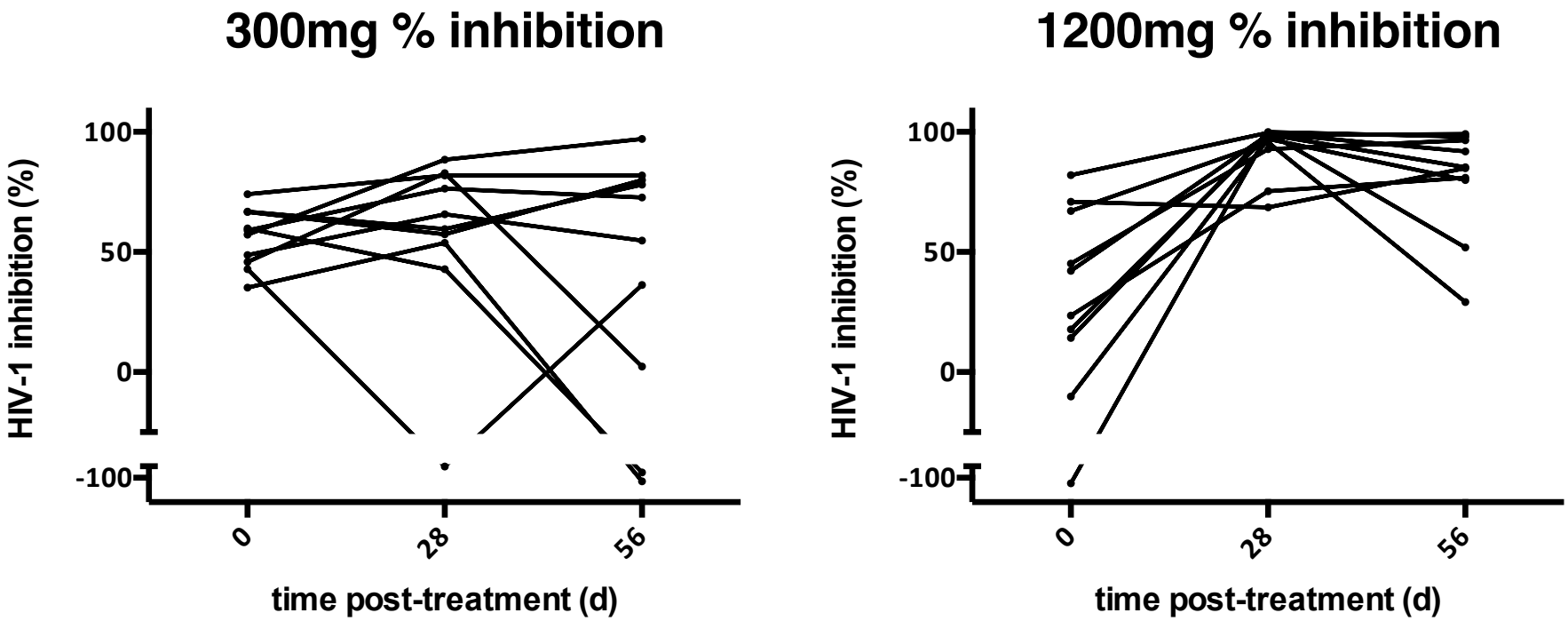


Figure 3:L HIV viral inhibition percentage measured ex vivo in cervicovaginal lavage in female participants at baseline and at 28 and 56 days postdose. (plotted lines from individual participants)

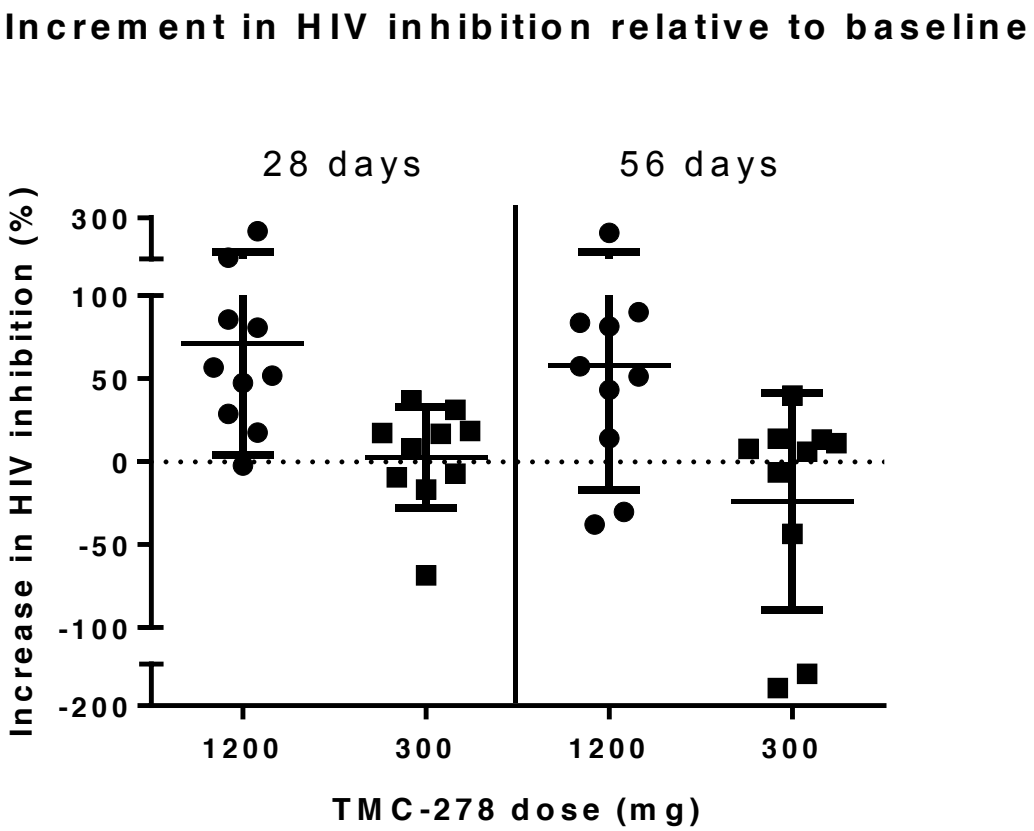


Figure 3:M Incremental change from baseline in HIV viral inhibition measured ex vivo in cervicovaginal lavage in female participants at baseline and at 28 and 56 days postdose.

3.4 DISCUSSION

This study was conducted to determine the concentrations of RPV in plasma, CVF, and VT in women after a single i.m. RPV-LA dose (300, 600, or 1,200 mg) and in the plasma, rectal compartment, and RT in men after 600 mg of RPV-LA i.m., over 84 days postdose.

Furthermore, the exploratory objective of this study was to investigate the effect of female genital-tract fluid (CVL) drug concentrations on HIV replication ex vivo, providing information on differential inhibitory effects of achieved RPV concentrations in biological fluids. The rationale for the study, study design, and dose selection and sampling intervals was based on the need to investigate the potential role of RPV-LA as an HIV PrEP agent and on the results from a previously conducted healthy volunteer study by the drug manufacturer (unpublished data, Janssen).

The study demonstrated that, after the administration of RPV-LA i.m. to both female and male healthy volunteers, measurable RPV plasma concentrations are achieved promptly (within 4 h post-administration) and persist for more than 84 days, in particular at higher doses. A secondary peak in the GM plasma concentration/time curves of the groups, observed at day 11, may reflect changes in the release of the drug from the injection site as well as complex RPV distribution kinetics (including immune cell trafficking of endocytosed nanoparticles from injection site to regional lymph nodes). It has been shown for long-acting preparations that the release profile can be influenced by changes in particle size

distribution as more dissolution occurs over time. However, multiple peaks were observed in the individual curves (as shown in

Figure 3:C); thus, the secondary peak appearing in the GM profiles may simply be due to increased relative sampling frequency during the first 14 days, as compared with thereafter.

RPV concentrations in the female genital tract (CVF and VT) are also achieved quickly and approximate those measured in plasma. Vaginal tissue concentrations were slightly lower than genital-tract fluid, possibly reflecting cell-to-fluid flux in response to concentration gradients.(Sugano et al. 2010; Van Damme et al. 2012; Mesquita et al. 2013) RPV is highly (~99%) bound to plasma proteins, primarily to albumin. Therefore, high accumulation of RPV in CV fluid cannot be explained solely by protein binding. Instead, physicochemical properties, including the molecule's dissociation constant, partition constant, and low molecular weight, may favour active transport and low clearance from the CV compartment. One explanation is that the more acidic environment of CVF (pH 4–5) as compared with either plasma (pH 7.4) or RF (neutral to slightly basic) means that a greater percentage of RPV exists in a protonated form (pKa (logarithmic acid dissociation constant)= 5.16), which may, in turn, result in mucosal "ion trapping" of the drug.

The RPV concentrations measured in the RT were equal to or higher than those in plasma, although RPV exposure in RF was approximately 75% lower than that in the plasma compartment. RF transudate may be diluted by luminal fluid derived from the proximal gut, potentially indicating that this sample may not be a representative

surrogate for RT concentrations with systemically delivered drug. Altogether, these data suggest a potential role of RPV-LA as a PrEP agent because of the exposures measured at the sites of HIV transmission.

Notably, target protective RPV concentrations in plasma or tissues are unknown and difficult to determine because of the lack of validated surrogate "efficacy" markers.

Therefore, potential efficacy markers are inferred from (i) animal models, (ii) human in vitro PD experiments, or (iii) phase III clinical trials of combination ARV treatment, including oral RPV. In the treatment trials, the mean RPV C_{trough} was 80 ng/ml, and the upper limit of the lowest quartile of exposures, in which group the virologic response was the lowest, was 50 ng/ml (unpublished data). However, a minimum effective RPV concentration in vivo for prevention has not been defined to date. It should be noted that the protein-corrected IC90 value (90% inhibitory concentration) for treatment is 12.1 ng/ml.(Azijn et al. 2010; van 't Klooster et al. 2010))

In the third stage of the study, CVL samples were collected to determine whether RPV present in the vaginal lumen would inhibit HIV infection of susceptible cells *ex vivo*. This well-established assay was previously used to assess the antiviral activity of CVL samples collected from women exposed to a tenofovir gel formulation.2,4,6,10 A limitation in quantifying drug in CVL is the unknown volume of vaginal fluid suspended in the saline wash; thus, it is not possible to derive a suitable correction factor for either drug or protein concentration. Nevertheless,

experimental analysis correlating concentrations achieved with such inhibitory effects suggests that a single dose of 1,200 mg should deliver sufficient drug to the genital tract to provide protection against sexual exposure to HIV; however, more data, including direct challenge of cervical biopsies as another marker of pharmacodynamic effect, are needed to confirm this.

We have also shown that BMI and gender may influence the absorption (Cmax) of parenteral RPV-LA. In particular, women with a BMI of >25 kg/m^2 may be susceptible to having lower RPV concentrations around the peak in the first 28 days after dosing, both in the systemic circulation and at the site of HIV transmission. For lipophilic agents such as RPV, the volume of distribution may be increased in subjects with high BMI, resulting in lower drug concentrations in both the central and peripheral compartments. However, these early findings are limited by an under-representation of men in the study cohort, and because BMI does not discriminate adipose tissue mass from muscular tissue, and men with high muscle mass are not well described.

Indeed, differences between men and women may be related to a combination of factors, including different rates of release of drug from the i.m. depot and the higher body fat percentage and smaller water content in women, apart from regional differences in adipose tissue distribution.

A further consideration is that in the female genital tract, changes in pH, stage of the menstrual cycle and level of mucus production, and permeability of the vaginal epithelium may also impact RPV-LA distribution in CVF and vaginal tissue, explaining

the high intra-individual variability observed in RPV concentrations in this compartment.

On the basis of these data, a phase I multiple-dosing study of RPV-LA for HIV prevention is currently under way investigating steady-state PK with repeated administration of 600 and 1,200 mg doses using viral inhibition in tissue explants (both genital and rectal) as a surrogate marker for efficacy. The consideration of these aggregated data, analysed by a population PK/PD model approach, will determine choice of dose magnitude and frequency in the design of a planned phase II study (assessing prophylactic efficacy in higher-risk populations) and, pending a favourable outcome, any subsequent global phase III studies. It is only during the latter two phases that a true determination of the efficacy and thus feasibility of parenteral HIV PrEP can be made.

ARV treatment depends on combination therapy, whereas current PrEP injectable agents are investigated as single drug prevention strategies because it is hypothesized that these would be sufficient to prevent HIV infection. However, delayed or missed injections could lead to prolonged periods of suboptimal drug exposure and increased risk of HIV acquisition; selective drug pressure in the presence of a replicating virus could enable expansion of drug resistance and onward transmission of resistant HIV strains. This may be especially true for nonnucleoside reverse-transcriptase inhibitors, in which single viral genotype mutations remarkably compromise the efficacy of this drug class,(Bannister et al. 2008) whose constituents have for several years been the most commonly used

1 third agents in first-line combination antiretroviral therapies.(World Health
2 Organisation 2013) 16

3 In conclusion, this study is the first to investigate RPV concentrations in the
4 female genital tract and male rectal compartment after the i.m. administration of
5 different RPV-LA doses to humans, and the first to assess the correlation between
6 compartmental drug exposure and HIV growth ex vivo. This study encourages
7 further development of RPV-LA as a potential PrEP intervention.

4 A CASE OF HIV SEROCONVERSION IN SSAT040

AGAJ, as the attending research physician, was responsible for the diagnosis, emergency management and ongoing follow-up of the patient described in this seroconversion case report. As Lead clinician on this parent study, AGAJ instituted the protocol-specified antiretroviral initiation plan for this patient and devised a bespoke schedule of comprehensive prospective sample collection, whilst putting in place an investigation to uncover the events leading to the proposal. AGAJ presented a report based on the investigation of these events to the protocol steering committee. On the basis of the ensuing discussion, a plan was enacted to engage the clinical academic capabilities of the Mellors' Group at the University of Pittsburgh, School of Medicine. In doing so, this new collaborative group was able to conduct a genotypic forensic examination of archived and prospective samples, reproducing in the lab the time course of the seroconversion. AGAJ coordinated this investigation and drafted the brief report, as second senior author, which was published in the Journal of Infectious Diseases, November 2015.

The aim of the study SSAT040 was to determine the tolerability and compartmental pharmacokinetics of a solid drug nanoparticle suspension of rilpivirine in plasma, whilst simultaneously determining its distribution to tissue sites of relevance to risk during sexual exposure to HIV infection; vagina and rectum.

As outlined in section 3.2.2, participants were systematically screened with a risk-based medical and sexual history in an attempt to enrol an healthy volunteer cohort, whose historical or current and likely future risk of HIV acquisition was minimised.

Despite this, unfortunately there was an occurrence of breakthrough HIV-1 infection in 1 female participant, enrolled in the 300-mg dose arm.

This was discovered only at the time of positive serology being confirmed from the final study sampling visit when she attended for follow-up. The participant was immediately recalled to clinic and received relevant clinical supportive care for a newly diagnosed patient. In addition and in line with the pre-specified protocol management pathway, empirical antiretroviral therapy with high genetic barrier combination of unexposed drug classes was initiated.

In agreement with the participant, now patient, a reconstruction of events from the her first study visit allowed her recall to isolate with a high degree of certainty the specific sexual encounter at which transmission is likely to have occurred. This was a single episode of vaginal intercourse with a new male partner, without the use of barrier contraception. The partner's HIV status, was unknown at the time of the encounter and he reported having a negative HIV antibody test within the three months prior. Though the partner was visiting the UK at the time of the event, he returned to his home country in southern Africa and was not available during the course of the follow-up of the index case.

This breakthrough infection was evaluated with the consent of the patient, allowing the collection and ongoing archive of follow up samples for at least six months after seroconversion, for plasma, serum and isolated peripheral blood mono-nuclear cells. We also obtained consent from the patient to engage the technical capability of a collaborating group at the Division of Infectious Diseases,

Department of Medicine, University of Pittsburgh School of Medicine, Pennsylvania allowing the anonymous transfer of data and samples for further investigation.

Herein, are reported the levels of RPV in plasma and cervicovaginal fluid (CVF) and the emergence of NNRTI-resistant HIV-1, with discussion of the potential impact of this resistance on subsequent treatment with first-line NNRTIs

The duration of TMC278LA detectability after injection has subsequently been formally investigated and demonstrated to exceed 150 days(McGowan et al. 2016), but the levels of active drug required to prevent HIV-1 infection have not yet been determined.

The prevalence of RPV-associated mutations is only 5% in treatment-naive individuals but is as high as 59% in patients with no response to NNRTI-containing first-line antiretroviral therapy (ART). (Anta et al. 2013; Lambert-Niclot et al. 2014; Parczewski et al. 2014; Theys et al. 2015)

The July 2014 International AIDS Society USA HIV-1 drug resistance mutation update recognised 17 mutations associated with RPV drug resistance, including L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, and M230I/L,(Wensing et al. 2014) which confer up to 50-fold resistance to RPV.(Azijn et al. 2010; Wensing et al. 2014; Basson et al. 2015)

During the ECHO and THRIVE trials, which evaluated RPV in a background of 2 NRTIs, E138K with M184I most commonly emerged in individuals with virologic failure. Of the samples from patients with virologic failure in these trials who

exhibited phenotypic resistance to RPV, 46% had virus with cross-resistance to nevirapine (NVP), 86% had virus with cross-resistance to efavirenz (EFV), and 91% had virus with cross-resistance to etravirine (ETR).(Cohen et al. 2013)

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4.1 Materials and Methods

SSAT040 study outline and conduct is reported above. Participants for the SSAT040 study were verified to be HIV seronegative by the 4th Generation GS HIV Combo Ag/Ab EIA HIV-1/2+O (Bio- Rad) prior to enrolment and were deemed to be at low risk for HIV infection as indicated by self-report. Plasma samples obtained at multiple time points from the participant with breakthrough infection were retrospectively tested for HIV-1 RNA (COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0, Roche Molecular Diagnostics) and for HIV-1 antibody at multiple time points, using the Bio-Rad 4th Generation GS HIV Combo Ag/Ab HIV-1/2+O EIA. Plasma and CVF were collected at regular intervals over 84 days for pharmacokinetic analysis. Resistance testing was performed on a residual sample collected following pharmacokinetic analysis. Sufficient plasma specimens for resistance testing were not available at all time points.

4.1.1 Resistance analysis

Resistance analysis was conducted in the laboratories at the University of Pittsburgh School of Medicine, by the Division of Infectious Diseases group led by John Mellors.

HIV-1 RNA from plasma samples was isolated using the ViroSeq 2.0 extraction module (Celera). In addition, RNA from plasma samples collected in heparinised tubes was precipitated in 3 M LiCl prior to amplification, to alleviate polymerase chain reaction (PCR) inhibition. Protease and full-length reverse transcriptase (amino acids 1 through 560) were amplified using in- house primers (OF1-BCD-5′-GAGGGACACCAAATGAAAG AYTG-3′ and 4232 5′-CCTGACTTTGGGGATTGTAGGGA AT-3′) in a 1-step reverse-transcription PCR, using SuperScript III One-Step (Invitrogen), followed by a second nested PCR, using in-house primers (Bcl1-5′-TAAGACAGTATGATCAAA TACTTATAGAAATTTGTGG-3′ and Xho1-5′-TAACTTTT CCCTCGAGATGTGTACAATCTAATTGCC-3′), to amplify full-length reverse transcriptase.

Sequence was generated by means of 6 bidirectional primers, using Sanger sequencing methods. HIV-1 drug resistance mutations were identified using the HIVdb program v7 (Stanford University) [14]. Allele-specific PCR for K101K and K101E was performed using RNA isolated for standard genotyping. Complementary DNA species were amplified and quantified in a first-round PCR, using Phusion Hot Start II High Fidelity Polymerase (Thermo Fisher Scientific) and HIV-1 subtype C reverse transcriptase– specific primers 28F 5′-AAACAATGGCCATTGACAGAAGA-3′ and 80R 5′-GTTCATACCCCATCCAAAGAAATG-3′.

A total of 1 × 106 copies of first-round PCR product were used in an allele-specific PCR reaction, using AmpliTaq Gold (Applied Biosystems) and the following patient-specific primers: forward wild-type primer 5′-GGAATACCGCACCCAGCAGGATTCA-3′, forward 101E primer 5′-GGAATACCGCACCCAGCAGGATT CG-3′, and reverse primer

5′-CTCTGGAATATTGCTGGTG ATCCTT-3′. The detection limit for K101E is 0.1%, based on testing a panel of K101:101E PCR amplicon mixtures included in each assay.

4.1.2 Phenotypic analysis

Full-length reverse transcriptase amplicons created for genotyping from the day 115 sample were used to generate recombinant patient-derived virus, using a modified HIV-1xxLAI vector [15]. PCR amplicons from the day 115 sample were cloned into Bcl1- Xho1 linearized xxLAI plasmids, using InFusion cloning technology (Clontech). DNA generated from single-colony bacterial isolates was selected based on having either K101 or 101E and having identical sequence for the remainder of the reverse transcriptase gene. Midiprep DNA from these isolates were used to transfect 293T cells, using Lipofectamine2000 (Invitrogen), and viral supernatants were collected 48 hours after transfection. Patient-derived viral stocks were used in drug susceptibility assays using TZM-bl cells and normalized for output of 100 relative light units in virus control wells. Fifty percent inhibitory concentrations (IC50) were determined for RPV, nevirapine (NVP), efavirenz (EFV), and etravirine (ETR).

4.2 Results

4.2.1 HIV-1 Infection and RPV Levels

One female participant in the SSAT040 study acquired HIV-1 infection through heterosexual intercourse without a condom with a new male partner approximately

41 days following receipt of a 300-mg single intramuscular injection of TMC278LA. The male partner was subsequently found to have acute HIV-1 infection at the time of transmission. Retrospective testing showed that the SSAT040 participant had undetectable plasma HIV-1 RNA (level, <100 copies/mL) on the day she received the TMC278LA injection (day 0). HIV-1 RNA was first detected on day 57 (level, 370 copies/mL), followed by seroconversion on day 84. The peak viremia level (644 925 copies/mL) occurred at day 115 and declined after initiation of ART with tenofovir/ emtricitabine and ritonavir-boosted darunavir (800/100 mg) on day 115 but remained detectable through day 275 (level, 125 copies/mL) until finally achieving viral suppression below the limit of quantitation (level, <40 copies/mL) on day 309 (Table 4:A, Figure 4:A). Both plasma and CVF drug concentrations peaked on day 15 (68.1 ng/mL and 294.9 ng/mL, respectively) and then dropped 6.5-fold and 16-fold respectively by day 41 (the suspected day of HIV exposure), but low levels of RPV were still detectable in plasma on day 226 (4.0 ng/mL). RPV drug concentrations were approximately 10.5 ng/mL and 18.3 ng/mL in plasma and CVF, respectively, at the time of HIV-1 exposure.

Table 4:A Rilpivirine Concentration, HIV Infection History and Drug Resistance Selection in a Seroconverter from the SSAT040 Study

Days post-IM injection[a]	[RPV] ng/mL			HIV Serostatus	Plasma HIV-1 RNA (c/mL)	CD4 (%)	Resistance Mutations[b]	% K101E[c]
	Plasma	CVF	Vaginal Tissue					
SCREENING				Neg				
0	0				<100			
0.17	4.08							
0.34	18.21	7.54						
1	37.42	51.28			<100			
4	53.62	91.83			<100			
7	58.16	133.25			<100			
11	42.6				<100			
15	68.11	294.89			<100			
22	24.34	135.31			<100			
28	24.33	32.89	243.23	Neg	<100			
44[d]	10.49	18.3			<100			
57	6.78	11.2	23.05	Neg	370			
84	7.51	13.97		Pos	175060		none	0
115[e]	4.05			Pos	644925	410 (22%)	K101EK[f]	19.4
122					74964	526 (34%)		
136					18006	692 (44%)		

Days post-IM injection[a]	[RPV] ng/mL	HIV Serostatus	Plasma HIV-1 RNA (c/mL)	CD4 (%)	Resistance Mutations[b]	% K101E[c]
151	13.75		6204	617 (49%)	K101EK	
175			2998	605 (49%)		
199	6.00		3558	834 (53%)	none	0.1
211			1184			
226	4.00		769			
275			125			
309			<40			

Abbreviations: IM, intramuscular; RPV, rilpivirine; CVF, cervicovaginal fluid; c/mL, copies/mL; neg, negative; pos, positive

[a] Participant was given a single dose of 300 mg TMC278LA.

[b] Resistance mutations were determined by standard genotyping.

[c] % K101E was determined by allele-specific PCR, with a limit of detection of 0.1%

[d] HIV exposure self-reported to occur on day 41. Day 44 samples are the first available after reported exposure.

[e] ART initiation with Truvada® and ritonavir-boosted darunavir (800/100mg)

[f] Susceptibility of d115 patient-derived recombinant HIV-1 isolates with K101E (HIV-$1_{d115/K101E}$) to RPV, nevirapine (NVP), efavirenz (EFV) and etravirine (ETR) was compared with d115 wild type HIV-1 isolates (HIV-$1_{d115/WT}$).
HIV-$1_{d115/K101E}$ had an RPV IC_{50} of 1.6 nM (4.3-fold resistance) compared to HIV-$1_{d115/WT}$ (IC_{50} 0.4 nM).
HIV-$1_{d115/K101E}$ had an NVP IC_{50} of 368 nM (7.9-fold cross-resistance) compared to HIV-$1_{d115/WT}$ (IC_{50} 46 nM).
HIV-$1_{d115/K101E}$ had an EFV IC_{50} of 2.8 nM (4.0-fold cross-resistance) compared to HIV-$1_{d115/WT}$ (IC_{50} 0.7 nM). HIV-$1_{d115/K101E}$ had an ETR IC_{50} of 4.0 nM (4.0-fold cross-resistance) compared to HIV-$1_{d115/WT}$ (IC_{50} 1.0 nM)

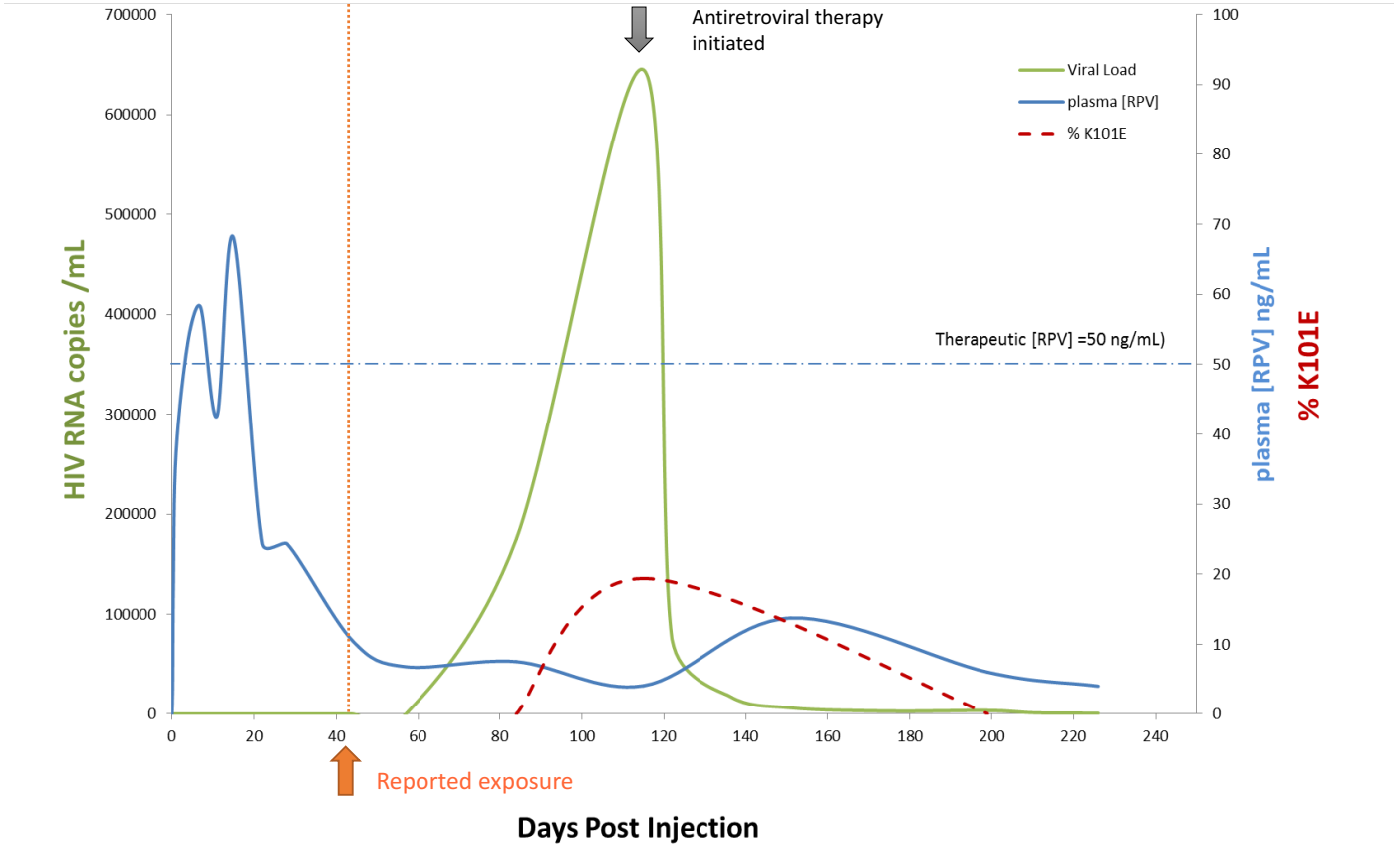
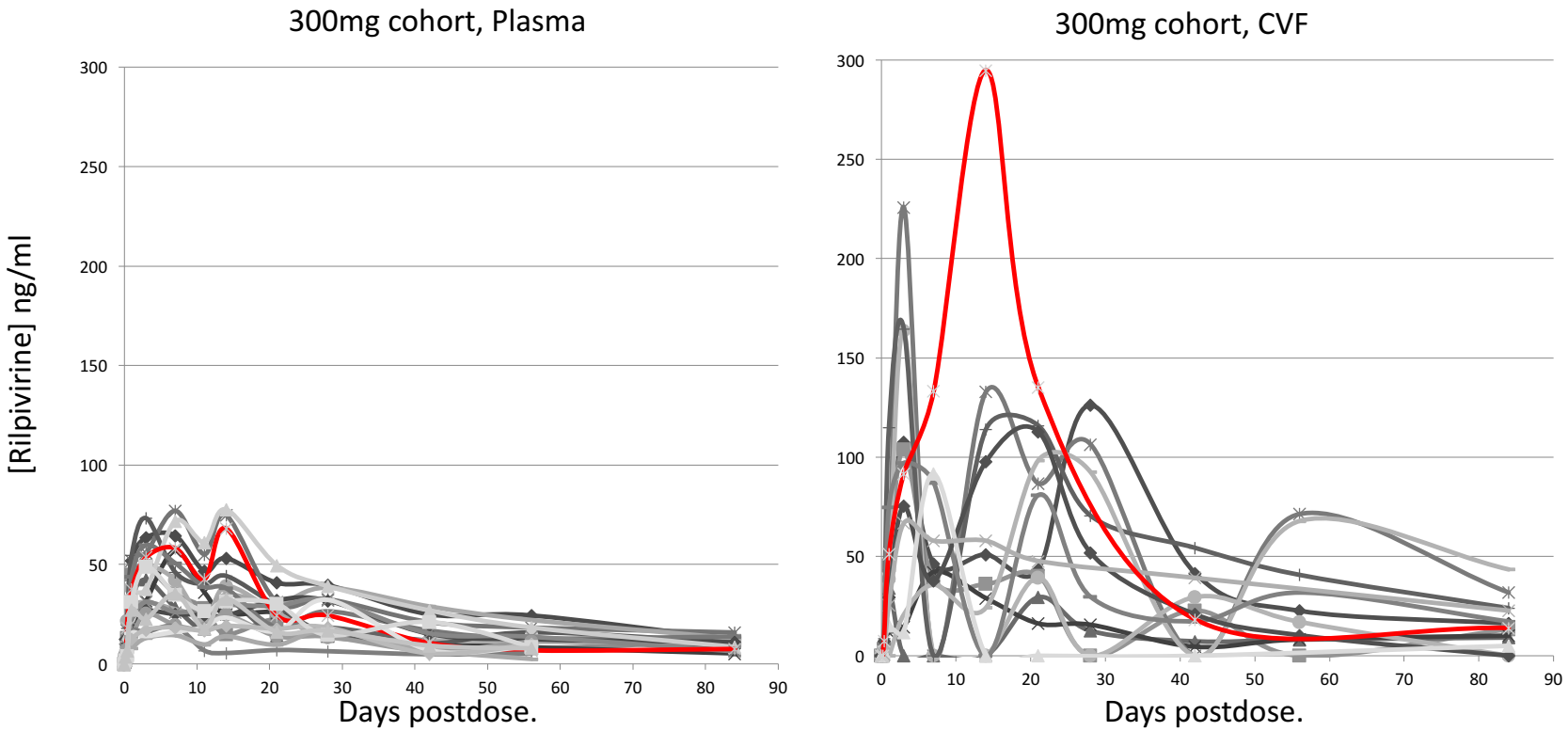


Figure 4:A Overlaid chronologic plot summarising HIV-1 RNA Copies/mL, Plasma Rilpivirine Concentration and Selection of K101E in seroconversion case.



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2 *Figure 4:B Individual plasma and CVF concentrations over 84 days following a single intramuscular dose of 300mg.*

3 Concentration plot for seroconversion case in red..

4.2.2 Selection of HIV-1 Drug Resistance

After evaluation of residual plasma samples, collected for pharmacokinetic analysis, for the presence of selected or transmitted drug resistance (Table 4:A), infection with wild-type virus, as determined by both standard population sequencing and allele- specific PCR with an assay sensitivity of 0.1% for 101E, was confirmed during acute infection on day 84. This was 43 days after the estimated date of HIV-1 infection. At the peak viremia level, on day 115, however, a mixed population of 101K/E was detected by both population sequencing and allele-specific PCR (19% 101E). By day 199, the predominant viral population was wild type, with only 0.1% 101E detected by allele-specific PCR (Table 4:A). Samples from the participant's sex partner were not available for testing.

4.2.3 Cross-resistance to NNRTIs

The day 115 patient-derived recombinant viral isolates containing either 101E or 101K was used to evaluate phenotypic resistance to RPV and cross-resistance to NVP, EFV, and ETR.

Day 115 isolates containing 101E had 4.3-fold greater resistance (IC50, 0.58 ng/mL) to RPV, compared with day 115 isolates containing K101 (IC50, 0.13 ng/mL).

Isolates containing 101E had 7.9-fold greater cross-resistance to NVP and 4.0-fold greater cross-resistance to both EFV and ETR (IC50, 98 ng/mL vs 12 ng/ mL for NVP, 0.88 ng/mL vs 0.22 ng/mL for EFV, and 1.8 ng/mL vs 0.44 ng/mL for ETR (Table 4:B)).

Table 4:B Seroconversion ex vivo phenotypic analysis

	RPV		NVP		EFV		ETR	
	Mean IC_{50} (ng/mL)	Fold Change	Mean IC_{50} (ng/mL)	Fold Change	Mean IC_{50} (ng/mL)	Fold Change	Mean IC_{50} (ng/mL)	Fold Change
K101K	0.13	-	12	-	0.22	-	0.44	-
K101E	0.58	4.3	98	7.9	0.88	4.0	1.8	4.0

Full-length reverse-transcriptase amplicons created for genotyping from the day (d) 115 sample were used to generate recombinant patient-derived virus using a modified HIV-1_{xxLAI} vector (see Materials and Methods).

Patient-derived viral stocks were used in drug susceptibility assays using TZM-bl cells and normalized for output of 100 RLU in virus control wells. Susceptibility to RPV, nevirapine (NVP), efavirenz (EFV) and etravirine (ETR) of d115 patient-derived recombinant HIV-1 isolates with K101E (HIV-$1_{d115/K101E}$) was compared with d115 wild type HIV-1 isolates (HIV-$1_{d115/WT}$) to generate fold-change resistance values.

4.2.4 Discussion

RPV concentrations in plasma and CVF after a single injection of 300 mg of TMC278LA were insufficient to prevent infection in 1 female participant from the SSAT040 study. The infection event occurred 41 days following TMC278LA administration, when the plasma RPV concentration was 10.5 ng/mL and the CVF RPV concentration was 18.3 ng/mL. Although the concentration of RPV required for prevention is not known, the RPV levels at the time of HIV exposure were well below the proposed target concentration of 50 ng/mL that is the minimum needed for virologic response when RPV is used as ART.(Cohen et al. 2013) Of note, the infected participant displayed higher peak concentrations of RPV in CVF and vaginal tissue, compared with other participants in the same 300-mg arm (who may or may not have had exposure to HIV), but free drug concentrations and the pharmacodynamic effect of RPV in these sites remains undefined.(McGowan et al. 2016) RPV levels continued to be low but detectable through the testing period of 226 days.

High levels of viral replication combined with low but detectable levels of RPV led to the selection of resistant variants containing K101E. Initial infection occurred with wild-type virus, as demonstrated by both standard and sensitive resistance testing, and thus resistance was selected by TMC278 and not transmitted from the participant's partner. A mixed population of viruses, consisting of approximately 80% wild-type and 20% mutant 101E variants, emerged by day 115, coinciding with peak viremia.

Phenotypic testing of the day 115 mutant showed resistance to RPV (4-fold) and cross-resistance to NVP (7-fold), EFV (4-fold), and ETR (4-fold), which indicates that the K101E mutant could negatively influence the virologic response to first-line

NNRTI-containing ART. The 101E variants declined to undetectable levels in parallel with the rapid decline in viremia level after ART initiation (Figure 4:A).

This case report is a unique instance of well-documented infection with wild-type HIV-1 and subsequent selection of resistant virus by continued drug exposure from a long-acting drug formulation. The 300mg dose of TMC278LA was insufficient to prevent infection and limit viral replication but was high enough to select RPV-resistant virus, illustrating a negative consequence of a long-acting antiretroviral formulation. RPV concentrations that prevent HIV infection prevention are undefined, but the 300mg dose of TMC278 did not achieve RPV levels above the proposed therapeutic concentration for virologic suppression (50 ng/mL). Further studies are needed to determine a target RPV concentration for HIV-1 prevention and to evaluate the risk of resistance during breakthrough infection with higher doses of TMC278. Both plasma and vaginal concentrations of RPV must be considered when selecting the appropriate dose for HIV prevention.

It is important to note the limitations inherent in the investigation of this seroconversion whilst receiving chemoprophylaxis which was insufficient to abrogate the establishment of infection. These limitations are inherent to the design of the primary study protocol, wherein the intention was to avoid HIV infection by selecting volunteer participants at low behavioural risk of infection for pharmacokinetic exploration, without testing pharmacodynamic efficacy of prevention of the investigational therapy.

Firstly, resistance testing was done using residual plasma specimens obtained after pharmacokinetic testing, so all intermediate time points were not available for standard or sensitive resistance testing.

Additionally, despite prolonged exploration of a variety of logistic means to allow the identified sex partner of the index case to consent to collection, processing and shipping of samples from his country of origin in Southern Africa, these repeated efforts were ultimately unsuccessful over a period of greater than 12 months. The objective of obtaining samples would have served two purposes. The primary importance, which became evident on discussion of the partner's access to clinical care within his home country, was that though therapy was accessible to him, this would likely be based on empirical initiation of a NNRTI-based antiretroviral therapy, as primary resistance testing would only be available at through commercial laboratories. Therefore, it would be important to confirm that he was indeed infected with wild-type virus based on the evidence of the investigation of the transmitted virus, rather than the harbouring the mutation seen as a minority species. Provision of this result to the patient would help to guide his choice, with a local healthcare provider, of initial therapy ensuring predicted full activity of all components. Secondarily, confirming his infection with wild-type virus, would corroborate the finding that the founder virus in the recipient was was wild type at codon 101, based on sensitive allele-specific PCR.

Nonadherence to daily or coitally dependent PrEP has emerged as the major barrier to PrEP effectiveness. Long-acting formulations have the advantage of infrequent dosing, but sustained, low drug concentrations for months after an

injection is problematic for the selection of drug resistance when breakthrough infection occurs. The post-hoc interrogation of the events occurring in this seroconversion highlights the importance of frequent HIV-1 testing with PrEP use and the need to develop a safe method for discontinuing long-acting products to avoid infection and drug resistance.

Elimination of rilpivirine is primarily through undergoing oxidative metabolism mediated by the cytochrome P450 (CYP) 3A system, with 85% recovery of radiolabelled dose in faeces and 6% in urine, with only trace amounts as the unchanged moiety (<1%). Given its high binding affinity to plasma proteins, predominantly albumin (99.7%), haemodialysis is unlikely to remove a significant proportion of the administered dose. In Table 4:A and Figure 4:A between the time of initiation of a ritonavir-boosted protease inhibitor combination on day 115 and the final PK follow-up sample on day 226, the plasma concentration of rilpivirine remains above 4 ng/mL across this period. Plasma concentrations at the two intervening (days 151 and 199) were 13.75 and 6.00 ng/mL respectively. The individual PK plots over time (Figure 3:C, Figure 3:E) illustrate a high degree of variability in sequential concentrations at interval, such that this apparent secondary peak in the presence of ritonavir may simply be sampling of the peaks and trough in that “tail” , rather than causally related to reduce elimination through cytochrome inhibition in the presence of ongoing release from the depot. It would not be unreasonable to infer that during the period of almost 4 months from day 115 period, the whole body clearance of rilpivirine was closely matched to the rate of release from the depleting depot. This raises a number of unanswered questions

with implications for registration phase study design and subsequent real-world use. Taking a base assumption that the 300mg dose has been demonstrated herein not to afford pharmacokinetic exposure able to sustain exposure above the putative target minimum threshold (50 ng/mL), planned future development phases have been able to eliminate, on the basis of futility, both the 300mg dose as well as higher doses which at predicted steady state, had concentrations at the end of the desired dosing interval lower than the above target threshold.

Thus most importantly, the dose of injectable long-acting formulations such as TMC278LA must be high enough and delivered at a frequency which enough to achieve drug levels that prevent HIV-1 infection.

5 SSAT049: Rilpivirine and Boosted-Darunavir Dual Therapy

AGAJ acted as Chief Investigator for this study and was responsible for designing the concept and securing industry funding in the form of an unrestricted grant, writing the protocol and leading the submissions for both ethical and regulatory approvals. AGAJ wrote standardisation manual and study report forms, as well as convening the Protocol Steering Committee to oversee the safe conduct of the study. AGAJ was responsible for the clinical care of all participants on study, including their recruitment from ambulatory clinics, obtaining informed consent and collecting clinical history and assessing physical examination and determining eligibility. Whilst on study, AGAJ was fully responsible for clinical management of these patients. AGAJ participated in the analysis of the bioanalytic output and presented the data as a poster at the 2014 Conference on Retroviruses and Opportunistic Infections and was lead author on the manuscript which has now been published in HIV Clinical Trials, 2018, (Jackson et al. 2017).

5.1 Background

Although combination antiretroviral therapy (cART) using three drugs, is recommended by international guidelines for the treatment of HIV infection,(Churchill et al. 2016) (Panel on Antiretroviral Guidelines for Adults and

Adolescents 2016) strategies which spare the use of drug classes, such as “dual therapies” in which combinations of 2 effective agents from different classes based on their mechanism of action, have been more recently been explored. There are a number of studies in which the putative aim of the avoidance of a drug class was to explore the potential to improve aspects of tolerability, adherence, and reduce observed toxicity and cost.

The NEAT001/ANRS143 study compared a combination of ritonavir-boosted darunavir 800mg/100mg once daily combined with either; raltegravir 400mg twice daily as the NtRTI-sparing dual-class regimen, or with tenofovir disoproxil fumarate/ emtricitabine 245mg/200mg once daily fixed-dose formulation as the standard triple class regimen in a randomised, non-inferiority comparison in previously untreated HIV-positive patients starting therapy for the first time in 15 European countries.(Raffi et al. 2014) The NrTRI sparing dual therapy was found to be non-inferior to standard therapy with treatment failure rates after 96 weeks of 17.8% and 13.8% respectively with a difference of 4.0%, with 95% confidence interval overlapping zero. The rate of occurrence of adverse events, classified as *serious* or which required modification of therapy, were similar between both groups. The expert study group came to the conclusion that this boosted-PI combination with integrase strand-transfer inhibitor (INSTI) could be a reasonable option for patients starting therapy with a CD4 count greater than 200 cells per μL.

Another head to head, open-label non-inferiority study conducted by the OLE/RIS-EST13 study group in 32 hospitals in Spain and France, compared the outcomes of patients already on a combination of ritonavir-boosted lopinavir,

combined with a two drug NRTI backbone containing either lamivudine or emtricitabine with another nucleotide/nucleoside.(Arribas et al. 2015) Within the study, 250 patients were randomised to either continue three drug therapy or to switch (reduce) to a combination of lopinavir/ritonavir 400mg/100mg twice daily with lamivudine 300mg once daily. Over 48 weeks after switching, the dual therapy rate of maintenance of treatment response was 87.8% (108/123), compared to 86.6% (110/127) in those remaining on three drugs. With non-inferior efficacy by statiscal tests and similar safety, in terms of adverse events and discontinuations, this combination of a boosted PI and NRTI was also demonstrated to be a suitable switch option.

More recently, a proof-of-concept pilot trial explored in a single-arm study design of 20 therapy naïve patients starting once-daily dual combination of the InSTI dolutegravir 50mg with lamivudine 300mg; the PADDLE study.(Cahn et al. 2017) All patients were screened for baseline genotype and started therapy with a viral load below 100.000 copies/mL (maximum 36,000 c/mL). Plasma viraemia was rapidly suppressed in all patients within the first 8 weeks, to below assay limits of quantification. Of the 20, one patient committed suicide and another experienced protocol-defined low level virologic failure at week 36, with return to suppression by the final visit of the study whilst remaining on therapy. This study is of particular interest as the dolutegravir and lamivudine (at these doses) are two components of the licensed single tablet fixed-dose once-daily combination (Triumeq®; dolutegravir 50mg/ abacavir 600mg/ lamivudine 300mg) (ViiV Healthcare 2014) but avoiding the potential toxicities which have been associated with the use of abacavir.

Within the aforementioned guidelines, cART containing two nucleoside reverse transcriptase inhibitors (NRTIs) plus a third agent are the most commonly recommended combinations (Churchill et al. 2016; Panel on Antiretroviral Guidelines for Adults and Adolescents 2016). However, long-term exposure to NRTI may lead to the development of adverse events with two of the most commonly used; tenofovir disoproxil fumarate(Gilead Sciences Inc. 2002) [potential long-term renal or bone toxicity] or abacavir sulfate(ViiV Healthcare 2000) [HLA-B*5701 related potentially severe hypersensitivity reactions and retrospectively observed increased risk of myocardial infarction].

Additionally, there are important considerations outside the immediate patient which are related to successes in treatment coverage which has co-evolved with secular trends in the public health epidemic.

I. the possible transmission of virus already containing resistance mutations, such that patients may have a viral genotype with less than optimal susceptibility to the commonly used NRTIs,(Baxter et al. 2015)
II. the low genetic barrier of certain NRTIs like lamivudine (3TC) and the likelihood of some patients to harbour resistance to this or other NRTIs if they have a history of poor adherence(Churchill et al. 2016) and
III. the rapidly increasing use of tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC)-base pre-exposure prophylaxis (PrEP) for HIV that may increase the development of resistance in individuals who seroconvert on PrEP,(Dimitrov et al. 2016), are other important factor to consider when investigating optimal alternative cART.

Consequently, there is importance in the evaluation of the of dual-therapy options, particularly where the patterns of use of NRTI-sparing may show an increased frequency of occurence.

Rilpivirine, is an antiretroviral agent approved in Europe and the USA for treatment of therapy-naïve HIV-1 infected adult patients (with a viral load </= 100,000 copies/mL) in combination with other agents. (Molina et al. 2012; Sanford 2012) A member of the non-nucleoside reverse transcriptase inhibitor (NNRTI) class, with a diarylpyrimidine structure, its activity mediated by non-competitive inhibition of HIV-1 reverse transcriptase. Rilpivirine is primarily undergoes oxidative metabolism mediated by the cytochrome P450 (CYP) 3A system, therefore drugs that induce or inhibit CYP3A may affect its clearance. The recommended dose is 25 mg given once daily, taken with a meal of at least 533 kcal.(Sanford 2012) (Janssen-Cilag Ltd 2011)

Darunavir, is a member of the protease inhibitor (PI) class. When co-administered with low dose ritonavir it is approved, in combination with other antiretroviral agents, for the treatment of patients with HIV-1. In treatment-naïve patients the recommended dose is 800 mg once daily with ritonavir 100 mg once daily taken with food.(Sherer 2007) (Janssen-Cilag Ltd 2015)

Ritonavir is a potent inhibitor of cytochrome CYP3A4 and when administered at a low dose of 100 mg once daily, it causes clinically significant increases in the plasma exposure of protease inhibitors and numerous other drugs processed by this common metabolic pathway.(Moyle and Back 2001)

A phase I study investigating the drug interaction between rilpivirine dosed at 150 mg once daily and darunavir/ritonavir in HIV-negative healthy volunteers showed an increase in rilpivirine pharmacokinetic parameters ranging between 80 to 180% with no changes in darunavir or ritonavir concentrations. (Santoscoy et al. 2008)

However, data in adult antiretroviral naïve patients living with HIV (PLWH) on the currently approved rilpivirine dose of 25 mg once daily are lacking. Yet, a combination of rilpivirine and ritonavir-boosted darunavir could potentially form a once daily NRTI-sparing treatment, as shown in a switch study (Maggiolo et al. 2016) and in a study in adolescents by. (Foca et al. 2016) Hence, this study investigated the antiviral activity, steady state pharmacokinetics and safety of rilpivirine plus darunavir/ritonavir in therapy-naïve HIV-infected adults.

5.2 Methods

5.2.1 Participants

Written informed consent was obtained from PLWH, aged 18 to 65 years old, naïve to cART with a VL > 1000 copies/mL and a CD4 count > 50 cells/mm3. Participants were excluded if they had significant acute or chronic psychiatric/medical illnesses that could have interfered with the ability of taking part

in the study, anomalies and risk factors for QTc prolongation or clinical laboratory determinations; positive screens for hepatitis B/C; baseline transmitted resistance compromising rilpivirine and darunavir efficacy; use of other drugs known to interact with the study drugs.

5.2.2 STUDY DESIGN

This was a single-arm, open-label study approved by the City and East Research Ethics Committee and the Medicines and Healthcare products Regulatory Agency (MHRA), UK (EUdraCT - 2012-002663-10; Clinicaltrials.gov NCT01736761).

Following recruitment of the first 10 participants with baseline viral loads below 100,000 copies/mL (group A), a protocol steering committee convened to review viral load responses over the first four weeks of therapy and to advise on whether to proceed with recruitment of participants with baseline viral loads above 100,000 copies/mL (group B).

After successful screening, participants were administered rilpivirine 25 mg plus darunavir/ritonavir 800/100 mg once-daily, and given weekly appointments until week 4 for drug concentration measurement (trough concentration, Ctrough), HIV-RNA testing and resting ECG monitoring. At week 4, they were admitted to the research unit for 24 hour pharmacokinetic sampling, blood was taken pre-dose, 1, 2, 4, 6, 8, 12 and 24 hours post-dose. Follow up appointments for safety laboratory

test, viral load measurement and ECG monitoring were at weeks 6, 8, 10, 12, 24, 36 and 48 post cART initiation.

On the pharmacokinetic day, the study medication was taken with a standardised breakfast (534 kcal) and 240 mL of water. Compliance with study drug administration was assessed by pill counting by the study staff throughout the study period.

5.2.3 Bioanalysis (drug plasma concentration measurement)

Blood samples were collected for the measurement of rilpivirine, darunavir, ritonavir concentrations into lithium heparin containing-blood tubes (six mLs) at each time-point, protected from light and immediately inverted several times and then kept on ice or refrigerated until centrifugation. Within 30 minutes of blood collection, each blood sample was centrifuged for 10 minutes at 1200 g at 4°C.

Plasma was then aliquoted equally into three 2.0 mL tubes (light protected) and stored at -20°C. Samples were shipped on dry ice to the Liverpool Bioanalytical Facility for analysis.

Plasma drug concentrations were determined using protein precipitation of analyte and stable isotope labelled internal standard using validated high-pressure liquid chromatography tandem mass spectrometry methods, as previously described [13,14]. The assay was validated over a calibration range of 15-15000

ng/mL (darunavir), 5-5000 ng/mL (ritonavir) and 0.5-400 ng/mL (rilpivirine). The accuracy (percentage bias) and precision (% coefficient of variation) were less than 15%.

5.2.4 DATA ANALYSIS

All HIV-RNA for the studied subjects were transformed to the Log10 scale and a linear mixed model fitted, with the trend estimated using a cubic spline. Furthermore, the mean of the decreases in Log10 viral load between successive visits have been compared for group A and group B at each visit time-point using a two-sample randomization t-test (R Foundation for Statistical Computing, Vienna, Austria).

The calculated pharmacokinetic parameters for rilpivirine, darunavir and ritonavir were the plasma concentration measured 24 hours after the observed dose (Ctrough), the maximum observed plasma concentration (Cmax) and the area under the plasma concentration curve from 0 to 24 hours (AUC0-24). All pharmacokinetic parameters were calculated using actual blood sampling time and non-compartmental modelling techniques (WinNonlin Phoenix, version 6.1; Pharsight Corp., Mountain View, CA).

Descriptive statistics, including geometric mean (GM) and 95% confidence intervals (95% CI) were calculated for all pharmacokinetic parameters. Inter

individual variability in drug pharmacokinetic parameters was expressed as a percentage coefficient of variation [CV, (standard deviation/mean) x 100].

5.3 RESULTS

Fifty-two PLWH and naïve to cART were screened for the study and 37 were enrolled. One subject withdrew for personal reasons, therefore 36 completed the study; baseline characteristics are summarised in Table 3. Thirty-five were males and 33 were Caucasian (two Indians and one Pakistani); median (range) age was 35 (21-58) years. Baseline median (range) CD4 count was 388 (170-1375) cells/mm3. Pre-cART resistance testing showed that no patient had any resistance to NNRTIs or protease inhibitors, while four had baseline resistance to NRTIs expressed as a single thymidine analogue mutation (TAM). Subjects with viral genotypes which included TAMs were re-questioned to confirm that their medical history did not include the use of antiretroviral medication as pre-exposure or post-exposure prophylaxis. All confirmed the absence of exposure to therapy whilst infected, and the TAM were assumed to have been transmitted at the time of infection.

1 *Table 3 Baseline demographic and clinical characteristics of the study participants*

	Group A (n=18)	Group B (*n* = 18)	All subjects (*n* = 36)
Gender (male:female)	17:1	18:0	35:1
Age in years (median [range])	36 [21 – 58]	35 [21 – 54]	34 [21 – 58]
Mode of transmission			
Men who have sex with men	16	17	33
Heterosexual	1	-	1
Unknown	1	1	2
HIV RNA (copies/mL), median [range]	91,999 [807 – 5,595,624]	24,403 [807 – 78,113]	215,120 [105,885 – 5,595,624]
HIV subtype			
B,(n)	15	13	28
Non-B, (n) Non-B subtypes were AG, D, C and AE	3	5	8
CD4 count (cells/mm^3); median [range]	388 [308 – 404]	450 [204 – 1375]	370 [170 – 787]
N with CD4 <200 cells/mm^3	1	None	1
QT_{cF} (msec); median [range]	360 [308 – 404]	358 [324 – 404]	360 [308 – 404]
Body Mass Index (kg/m^2); median [range]	23 [16 – 35]	22 [20 – 28]	23 [16 – 35]

5.3.1 VIRAL LOAD DYNAMICS

Eighteen patients had a baseline viral load below 100,000 copies/mL (group A) and 18 with a baseline viral load above 100,000 copies/mL (group B) at screening (Table 1). Viral load decay curves are illustrated in (Figure 5:A, Figure 5:B, Figure 5:C).

All but one (viral load = 63 copies/mL) study patients achieved viral load < 50 copies/mL by week 36 and all by week 48 (Table 1). Overall median (range) viral load reduction (Log10 copies/mL) was 1.3 (0.6-1.9) over the first week of treatment, with no major differences between group A and B (Table 5:D).

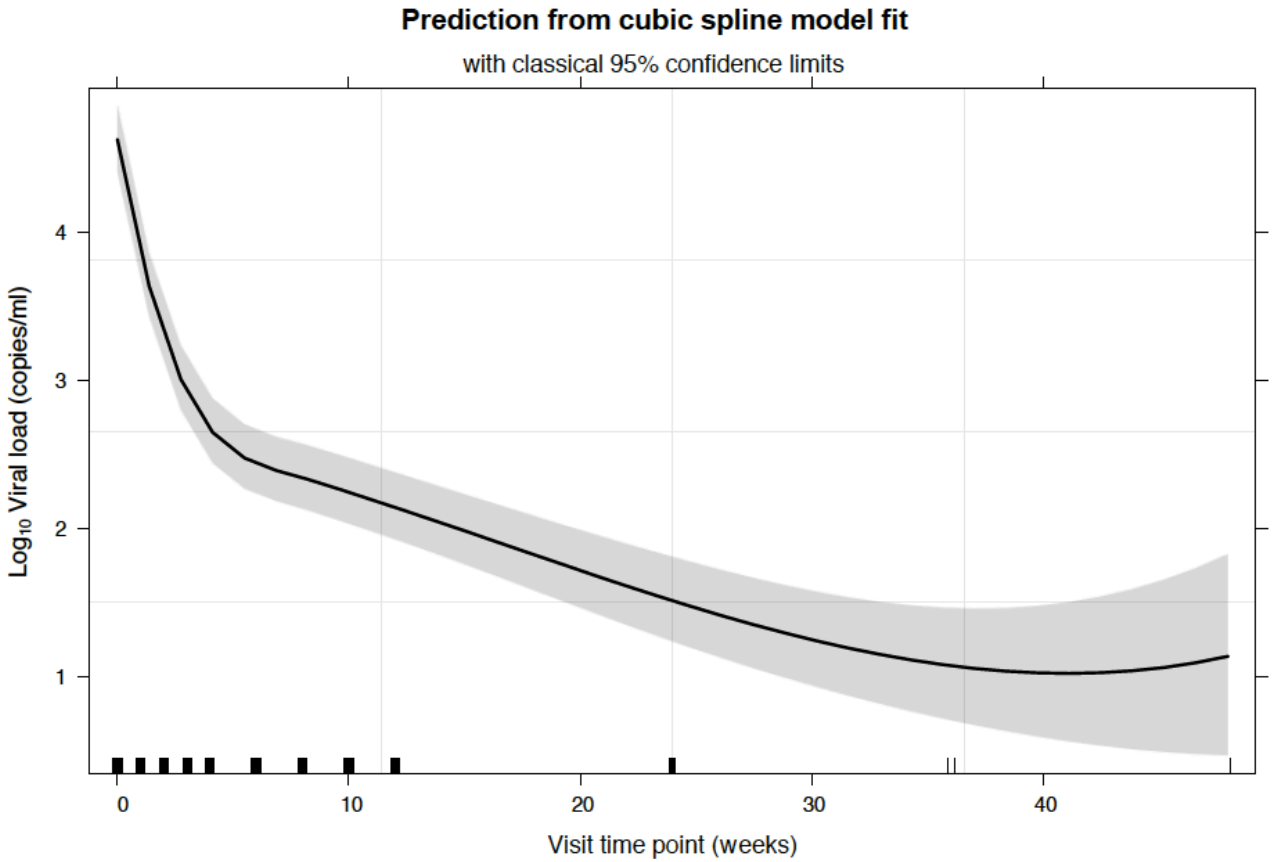


Figure 5:A Geometric mean viral load decay over 48 weeks treatment with rilpivirine/ darunavir/ ritonavir in 36 study patients.

Table 5:D Viral load (VL) decay in the 36 patients who completed the study, and in group A (VL < 100,000 copies/mL) and group B (VL >/= 100,000 copies/mL) over the study period.

Between visit Log_{10} viral load decrease (copies/ml)	Subgroup of HIV RNA at baseline								
	Overall			**U = under 100,000 copies/mL**			**A= At least 100,000 copies/mL**		
	N = 36			N = 18			N = 18		
	n	Median	IQR	n	Median	IQR	n	Median	IQR
Screening to baseline	36	**-0.05**	-0.2 to 0.2	18	**0.12**	-0.2 to 0.2	18	**-0.14**	-0.2 to 0.0
Baseline to wk 1	36	**1.30**	1.2 to 1.6	18	**1.30**	1.1 to 1.5	18	**1.35**	1.2 to 1.7
Wk 1 to wk 2	34	**0.36**	0.2 to 0.6	17	**0.35**	0.2 to 0.4	17	**0.39**	0.2 to 0.7
Wk 2 to wk 3	34	**0.17**	0.1 to 0.3	17	**0.17**	0.1 to 0.4	17	**0.17**	0.1 to 0.3
Wk 3 to wk 4	32	**0.14**	0.0 to 0.2	15	**0.03**	-0.1 to 0.2	17	**0.19**	0.0 to 0.2
Wk 4 to wk 6	31	**0.24**	0.1 to 0.4	14	**0.27**	0.0 to 0.5	17	**0.24**	0.1 to 0.4
Wk 6 to wk 8	30	**0.19**	0.0 to 0.4	12	**0.01**	-0.1 to 0.1	18	**0.28**	0.2 to 0.4
Wk 8 to wk 10	27	**0.25**	0.0 to 0.4	9	**0.25**	0.1 to 0.4	18	**0.18**	0.0 to 0.4
Wk 10 to wk 12	26	**0.17**	0.0 to 0.3	9	**0.16**	-0.0 to 0.3	17	**0.18**	0.0 to 0.5
Wk 12 to wk 24	6	**0.73**	0.6 to 1.1	0	-	-	6	**0.73**	0.6 to 1.1
Wk 24 to wk 36	1	**0.03**	0.0 to 0.0	0	-	-	1	**0.03**	0.0 to 0.0
Wk 36 to wk 48	0	-	-	0	-	-	0	-	-

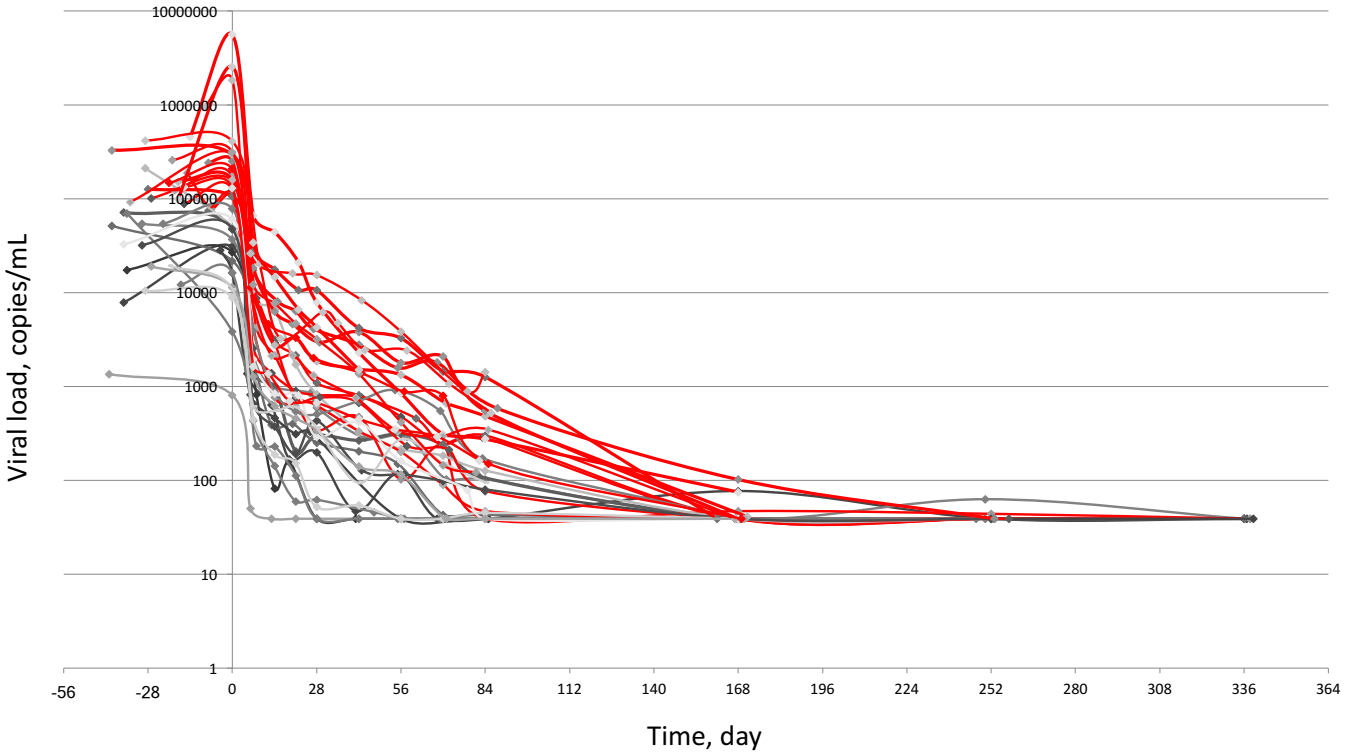


Figure 5:B Individual patient viral load kinetics over 48 weeks treatment with darunavir/ritonavir/rilpivirine (baseline VL over 100,000 copies/mL in red)

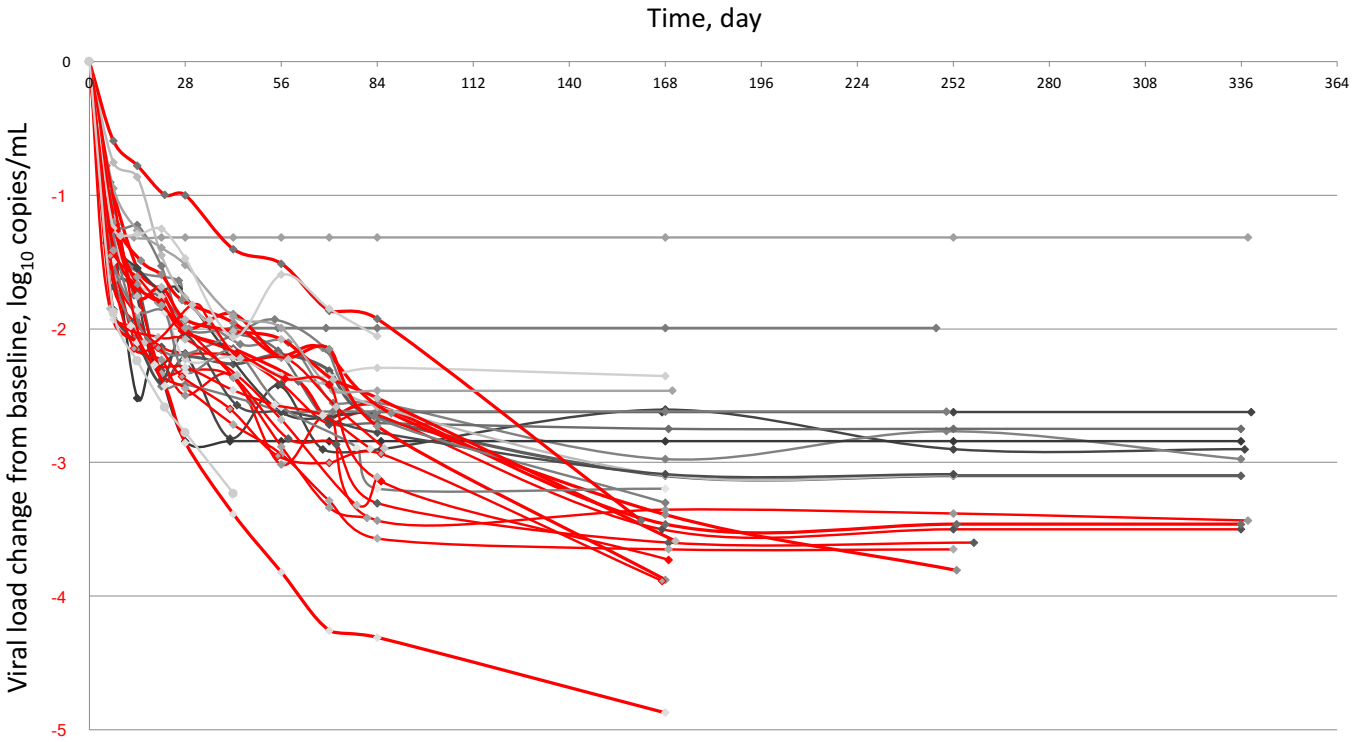


Figure 5:C Individual patient viral load kinetics (delta from baseline0 over 48 weeks treatment with darunavir/ritonavir/rilpivirine (baseline VL over 100,000 copies/mL in red)

5.3.2 PHARMACOKINETICS

GM plasma concentration versus time curves for rilpivirine, darunavir and ritonavir are shown in (Figure 5:D,Figure 5:E) and their pharmacokinetic parameters summarised in Table 5:E.

Although approximately 45% higher, rilpivirine AUC measured in this study was within the range of those reported from Phase III pharmacokinetic sub-studies.(Molina et al. 2012) All subjects had darunavir Ctrough values above the protein binding adjusted IC50 of 550 ng/mL, and the darunavir pharmacokinetic parameters measured were similar to those reported from Phase III studies (Table 5:E).(Sherer 2007)

Table 5:E Rilpivirine, darunavir and ritonavir pharmacokinetic parameters over a 24 hour dose interval at steady state in 36 patients living with HIV, expressed as geometric mean and 95% confidence intervals.

PK parameter	Rilpivirine	Darunavir	Ritonavir
N = 36	Geometric mean (95% C.I.)		
AUC_{0-24h}	**3036**	**82598**	**4455**
ng.h/mL	(2876 – 3969)	(76508 – 113143)	(4098 – 64752)
CV%	49	59	68
C_{max}	**188**	**8381**	**503**
ng/mL	(175 – 248)	(7802 – 11279)	(463 – 845)
CV%	53	56	90
C_{trough}	**116**	**1728**	**42**
ng/mL	(106 – 171)	(1661 – 2669)	(38 – 60)
CV%	72	71	68

AUC_{0-24h} ; area under the curve between 0 to 24 hours. C_{max}; maximum concentration. C_{24h}; concentration at 24 hours. CV ; coefficient of variation

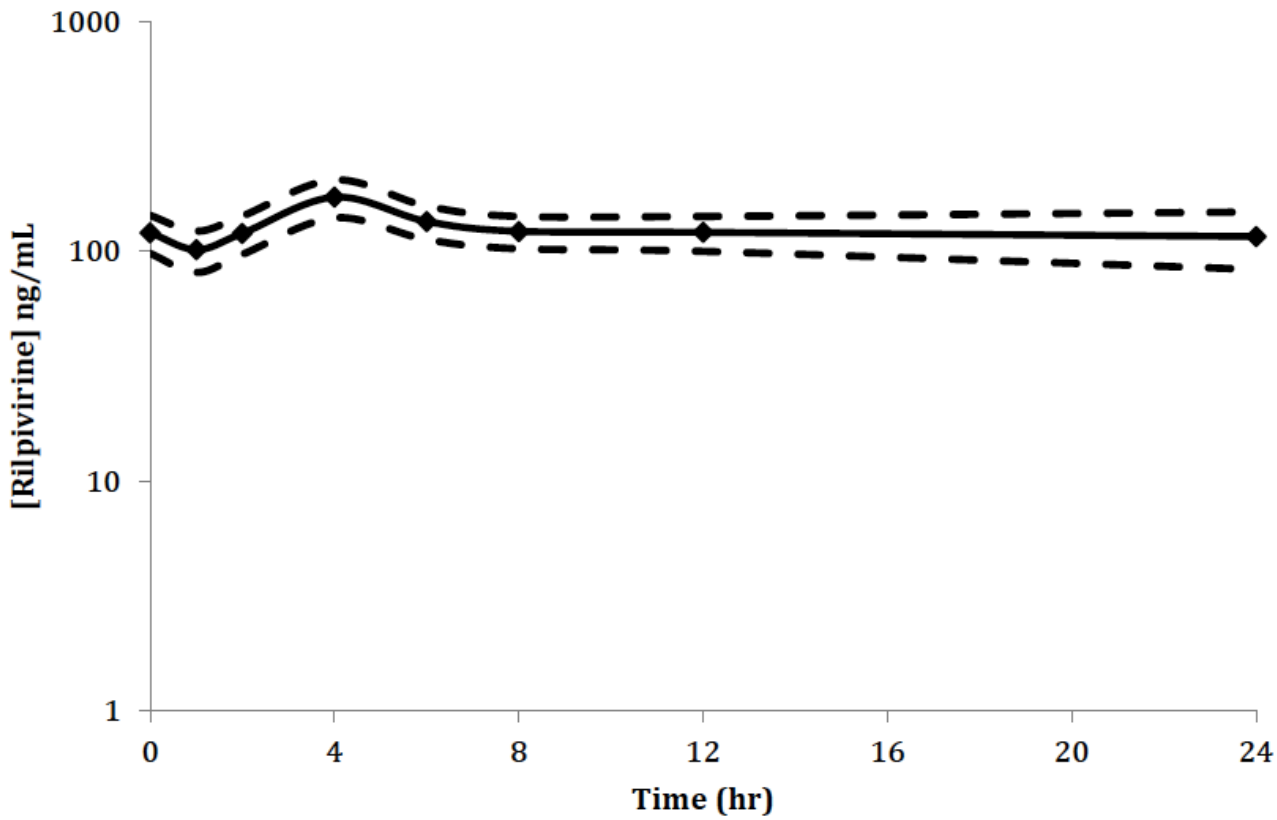


Figure 5:D Geometric mean (GM, solid line) and 95% confidence interval (95%C.I., dotted lines) plasma concentrations of rilpivirine.

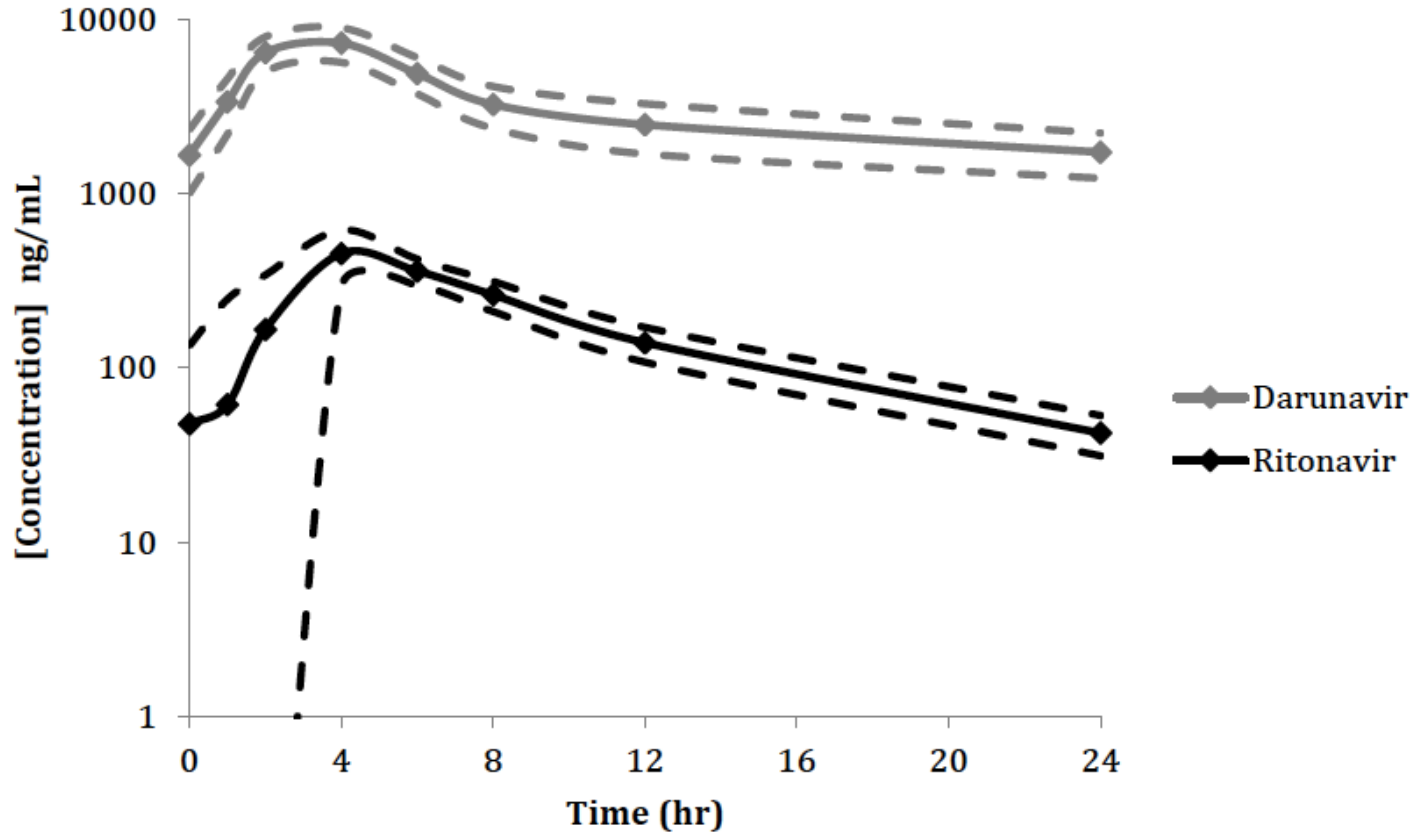


Figure 5:E Geometric mean (GM, solid line) and 95% confidence interval (95%C.I., dotted lines) plasma concentrations of darunavir/ritonavir.

5.3.3 Safety and tolerability

Study drugs were well tolerated with no subjects discontinuing rilpivirine and darunavir/ritonavir because of drug related toxicity. Three serious adverse events were recorded during the study, none of which were deemed by the investigator to be related to study medication: i) admission to hospital for treatment of prostatitis complicated by peri-urethral abscess; ii) overnight admission to hospital to treat dehydration and confusion caused by recreational drug intoxication; iii) laparotomy and bowel resection with temporary stoma to repair rectal trauma.

There were no other adverse events higher than Grade 2 of severity. Two patients experienced a transient self-limiting generalised rash (without mucosal involvement) following cART initiation, which resolved with continued dosing.

No significant change from baseline and no QTcF interval greater than 450 msec were recorded during the study and no laboratory parameter abnormalities of greater severity than Grade 2 were measured.

5.4 Discussion

In this pharmacokinetic/pharmacodynamic study in HIV-1-infected treatment-naive participants, the NRTI-free combination containing rilpivirine and

darunavir/ritonavir led to achievement of an undetectable viral load in all subjects at week 48 (and all but one who had a viral load of 36 copies/mL, by week 36). No differences in viral load decrease or achievement of an undetectable viral load were observed between patients with a baseline viral load above and below 100,000 copies/mL.

Rilpivirine exposure was slightly increased by ritonavir co-administration via CYP3A4 inhibition; however, its AUC was still within the range observed in historical controls.(Molina et al. 2012) Importantly, no changes in ECG QTcF were seen during the study. The study drugs were well tolerated with no drug related adverse events or laboratory parameters higher than Grade 2.

While an association between efficacy and baseline viral load has been demonstrated with other cART combinations containing two or three active drugs,(Sax et al. 2009; Taiwo et al. 2011; Raffi et al. 2014) this small but intensive study showed that rilpivirine plus darunavir/ritonavir is efficacious independently of baseline viral load. A potential explanation of this finding could be the slightly higher concentrations that were measured for rilpivirine in the presence of darunavir/ritonavir or the fact that being a pharmacokinetic/pharmacodynamic study, participants were followed closely and adherence monitored frequently, in particularly during the first four weeks of treatment in which this was conducted weekly.

As rilpivirine doses three times higher than the recommended dose of 25 mg once-daily can prolong the QTc interval,(Sanford 2012) and an increase in rilpivirine

concentrations was expected because of ritonavir co-administration and therefore inhibition of rilpivirine metabolism. ECG recordings throughout the study were available and showed no change in QTcF from baseline. Therefore, no correlation between QTcF change and rilpivirine C_{max} could be calculated.

Dual antiretroviral treatments containing a boosted protease inhibitor-based therapy, (e.g. a boosted protease inhibitor plus one agent from another class) is not the preferred strategy recommended by most recent guidelines. However, over the past 15 years numerous studies have been published showing how dual antiretroviral therapies may be beneficial in clinical practice. Importantly, a recent meta-analysis showed minor differences in terms of virological efficacy when comparing dual to triple cART. (Achhra et al. 2016)

Furthermore, it was observed that although a similar risk of serious adverse events in patients on dual and triple therapies, the former had a lower risk of adverse events leading to discontinuation, suggesting that dual therapies may be better tolerated. (Achhra et al. 2016)

Interpretation of this study should take into account its single-arm design and the small number of patients studied, and a larger randomized trial is warranted to draw definite conclusions. However, interestingly in the past, single arm Phase IIb trials have predicted more definitive findings than those in Phase III clinical trials: the ACTG A5262 study was followed by NEAT001 that showed that the dual regimen containing raltegravir plus darunavir/ritonavir did not achieve non-inferiority in individuals with high baseline viral loads and low CD4 counts.(Taiwo et al. 2011; Raffi

et al. 2014) Beyond this study, the efficacy of RPV/DRV/r in maintaining an undetectable viral load in suppressed patients as a switch strategy and in adolescents has also been confirmed.(Foca et al. 2016; Maggiolo et al. 2016)

There is increasing interest from healthcare practitioners involved in management of HIV patients in routine practice, outside academic clinical studies, in the use of reduced number of drugs used in standard antiretroviral regimens. A variety of different innovative combinations, which avoid the use boosted protease inhibitors, are being studied utilising drugs with a high genetic barrier such as dolutegravir, in combination with an NRTI (e.g. lamivudine, 3TC)(Cahn et al. 2017) or an NNRTI (e.g. rilpivirine). {Llibre:2017uh} The outcome of these studies is important because new dual combinations may limit drug-related toxicities in both early and long-term antiretroviral use. Importantly, however, dual regimens need to be carefully selected and to date large randomised clinical trial data on dual therapy are available only for boosted protease inhibitor-containing combinations.(Cahn et al. 2014; Arribas et al. 2015; Cahn et al. 2017)

To summarise, this study has demonstrated that the combination of rilpivirine and darunavir/ritonavir shows efficacy in therapy-naïve HIV infected patients and is generally well-tolerated without exhibiting any safety signals of concern. Given this, it is potentially useful as an alternative strategy in patients for whom standard cART is not an option due to resistance or toxicity.

6 SSAT055: NANO-EFAVIRENZ AND NANO-LOPINAVIR

AGAJ helped to design this study and wrote the first drafts of the protocol, leading on the steps to obtain regulatory and ethical approval. In particular, the candidate was responsible for the design of efavirenz-containing arms of this study, by ensuring that in accordance with the European Medicines Agency guidance on bioequivalence, they would potentially fulfil the minimum requirements necessary for registration purposes. The study was delayed for logistic reasons, and resumed only after AGAJ had changed employment and was no longer clinically responsible for study conduct. However, on resumption of study activities, AGAJ served as a member of the protocol steering committee, and conducted the pharmacogenomics analyses on samples obtained from volunteers in the study.

6.1 BACKGROUND

Chapters 3 and 4 of this manuscript, explore the use of a solid drug nanoparticle (SDN) suspension of a non-nucleoside reverse transcriptase inhibitor. This study served as a work of conceptual proof, in determining the potential utility or (and) futility of administering the active pharmaceutical ingredient, rilpivirine, parenterally to an intramuscular site to act as a pre-exposure agent for the prevention of HIV infection.(Jackson et al. 2014; Penrose et al. 2015)

The strategic approach of manipulating an existing active pharmaceutical ingredient (API) into a new formulation presents an appealing opportunity. The ability to modify a poorly aqueously soluble drug as nanometer scaled particles presents a potentially advantageous means of modifying the physiological and chemical properties of these drugs and their interaction with either enteral or parenteral absorptive tissues and organs, by enhanced absorption and distribution kinetics. This, whilst at the same time using a lower total amount of API with lower per administration doses, reduced frequency of dosing and potentially lower peak post-absorptive concentrations with prolonged therapeutic coverage.

Several nanomedicine strategies can be hypothesised to improve drug delivery and, in general, polymer materials are used in main approaches underpinning the science of nanomedicines. SDN are structured as stabilised particles, with each particle composed entirely of the active drug, unlike the variety of nano-scale formulation approaches which utilise a nano-carrier as a vehicle for the active moiety (polymer micelles, liposomes or vesicles, polymer particles, or drug-polymer conjugates).

The advantage of creating SDN using products which have previously been developed through registrational clinical trial programmes, thus achieving full marketing authorisation with well-defined efficacy, tolerability and safety in standard formulation, allows some simplification of the route to a viable licensed product.

In the case of rilpivirine (discussed in chapters 3 and 4) this transition and repurposing, by adopting a new mode of delivery from an orally ingested tablet for enteral absorption to a parenteral long-acting intramuscular depot, is not simply a matter of demonstrating bioequivalence between routes of administration. A range of new questions arose during the course of its development. There were physio-chemical factors relating to the stability of the new formulation, its impact on quality of the manufactured product and the resilience of the product real-world use. Additionally, there were patient and healthcare provider considerations of acceptability and reliability of such a product as well as how to manage adverse reactions with a non-reversible product with no current means of removal once administered.

Even beyond this, these sets of challenges questions do not cover the range of host-related factors which may differ between the use of an API in established infection, in comparison to how the API must interact with the range of host susceptibility factors from the whole-body systematic level through to local organ and tissue specific innate protective mechanisms at rectal and genital sites and their interaction with local vaginal and gut flora.

The suspension-based antiretroviral products which have been studied to date for intramuscular injections (rilpivirine and cabotegravir – an integrase inhibitor being developed by ViiV Healthcare) have typically utilised particles whose average diameter is <10μm which are created through mechanical attrition processes such as nano-milling and high-pressure homogenisation, often termed a “top-down” approach. This is a batched process, which uses macroscopic solid form API as a

starting material and within a sterile contained system, gradually reduces the size of the solid by continuously grinding the API against spherical small-diameter ceramic beads over a prolonged period of time. The size and uniformity of the of the desired SDN are time dependent, with greater durations required for smaller particles within a more narrow range of particle size.

For example, the G001 formulation of rilpivirine under current development by Janssen is a sterile suspension is based on NanoCrystal® technology, involving a wet bead milling process (NETZSCH Lohnmahltechnik GmbH, Bobingen, Germany) to produce particles of pure rilpivirine of average size 200nm over a milling duration of 50 days.

A novel alternative to nano-milling for the production of SDN is the “bottom-up’ approach using an innovative emulsion-templated spray-dry manufacturing process. (Zhang et al. 2008; Grant and Zhang 2011) In this bottom-up approach, the drug nanoparticles are formed from molecules in an emulsion. The emulsion is freeze-dried by spray freezing into cold liquid to prepare a dry SDN material which can either be readily re-suspended in aqueous medium, forming a nano-particulate suspension, or packaged into capsules for oral administration. To date, a series of candidate SDN antiretrovirals have been generated at the University of Liverpool, formerly in collaboration with IOTA NanoSolutions Ltd (Liverpool, UK) and supported by the Research Councils UK and Engineering and Physical Sciences Research Council funding.

This study will explore the pharmacokinetic exposures obtained in HIV-negative healthy volunteers, with oral nano-formulated versions of two existing approved antiretroviral agents (efavirenz and lopinavir) which have been synthesised to GMP standards using the Liverpool technology. These will be compared to the exposures obtained with the existing marketed formulations (Sustiva® - efavirenz and Kaletra® - lopinavir/ritonavir) with the ultimate aim of determining a dose of each nanoformulation which is bioequivalent to the licensed product.

For Kaletra® the comparative dose will be the 400/100mg (2x 200mg/50mg film coated tablets) licensed twice-daily dose for treatment of HIV-infected adult patients.

For Sustiva®, {BristolMyersSquibbPharmaceuticalsLimited:2002ww} the aim is to study both the licensed dose of 600mg once a day {sustiva smpc ref} and a lower dose of 400mg (2 x 200mg capsules). This lower dose is based on the published data within the ENCORE-1 study (clinicaltrials.gov identifier NCT01011413), …… , which showed matched effectiveness of the daily 400mg dose to 600mg of efavirenz, when dosed daily with tenofovir disoproxil fumarate/ emtricitabine 245mg/200mg once-daily fixed dose combination.

6.2 METHODS

This study is a phase I, open-label prospective pharmacokinetic study investigating two new antiretroviral formulations - NANO-efavirenz (NANO-EFV) and NANO-lopinavir (NANO-LPV) in parallel and employing an adaptive design with two stages. The results obtained in the primary stage are used to inform the NANO-antiretroviral doses selected for investigation in the secondary stage.

The primary objective of the first phase was to investigate the pharmacokinetics of NANO-EFV and NANO-LPV in HIV-negative healthy volunteers after receiving a single dose and after multiple dosing at steady-state.

6.2.1 PARTICIPANTS

The study was conducted within clinical research ward facility of the St Stephen's Centre, Chelsea and Westminster Hospital in London, UK. Written informed consent was obtained from male and non-pregnant, non-lactating female healthy volunteers recruited through a database of volunteers and NRES-approved advertisement within the local Trust. Volunteers were aged between 18 to 65 years, inclusive with a recorded body mass index (BMI) from 18.0 to 30.0 kg/m^2. Participants were screened by medical history (confirmed with their GP), physical examination and screening laboratory assessments and were excluded if they had any significant acute or chronic psychiatric or medical illness, any prescribed or over-the-counter use of medication, electrocardiographic anomalies or QTcF $\geq$ 450 msec, elevated

transaminase (ALT and AST) > 1.25 times the upper limit of normal, positive serology for a bloodborne virus (HIV, Hepatitis B or C), recreational drug use (by history and urine drug screen), any known personal or family history of cardiac disease or sudden cardiac death, participation in a clinical trial of an investigational medicinal product within 3 months prior to screen date, and for females of childbearing potential a positive pregnancy test at any point at or after screening.

6.2.2 Pharmacogenomics

For both stages, in all groups and all arms, volunteers were invited to consent to a separate sub-study in which a single plasma sample was collected at any point on or after day 1, de-identified through a linked anonymised protocol and stored and shipped to the University of Liverpool for later analysis for gene polymorphisms relevant to the metabolic disposal of the studied drug.

6.2.3 Bioanalysis (Drug plasma concentration measurement)

Blood samples were collected for the measurement of efavirenz, lopinavir and ritonavir concentrations from an indwelling catheter, or direct venepuncture if not available, at each sampling time. Six mLs of whole blood was collected into an evacuated lithium heparin blood collection tube at each timepoint, immediately

inverted several times to mix and placed on wet ice or refrigerated until centrifugation, within 90 minutes of collection. Each sample was spun for 10 minutes at *2000 g* at 4 °C and then equal aliquots transferred to three opaque polypropylene capped storage tubes for storage at -20°C or lower until shipping to the pharmacology research laboratories at Liverpool. Concentrations of efavirenz, lopinavir and ritonavir in plasma were measured using a validated HPLC – tandem mass spectrometry method. (Else et al. 2010; Amara et al. 2011) with a lower limit of quantification of 25 ng/mL for efavirenz and 75 ng/mL for lopinavir.

6.2.4 Bioanalysis (Pharmacogenetic Analysis)

Whole blood was collected in a 4mL evacuated EDTA collection tube from those participants who gave written informed consent to the substudy. Samples were deidentified with a secondary unlinked code and the sample was stored at 20°C until shipping to the pharmacology research laboratories at Liverpool. Genomic DNA was extracted from a 250μL aliquot of thawed whole blood whole blood through use of the manufacturers protocol (E.Z.N.A Blood DNA Mini Kit; Omega biotek; Nor-cross, GA). Extracted DNA was quantified using NanoDrop (Thermo Fisher Scientific, Wilmington, DE). Genotyping was completed using real- time allelic discrimination polymerase chain reaction (PCR) assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA). The PCR protocol followed denaturation at 95°C for 10 min, followed by 50 cycles of amplification at 92°C for 15 sec and annealing at 60°C

for 1 min 30 sec. Taqman Genotyping Master mix and assays were purchased from Life Technologies (Paisley, Renfrewshire, UK). Opticon Monitor v. 3.1 software (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and identify genotypes.

Table 6:A Pharmacogenetic single nucleotide polymorphisms assayed for participants in each treatment group.

Efavirenz	Lopinavir
CYP2B6 516G>T (rs3745274)	CYP3A4*22 99366316G>A (rs35599367)
CYP2B6 983T>C (rs28399499)	CYP3A5*3 6986A>G (rs776746)
SLCO1B1 521T>C (rs4149056)	SLCO1B1 521T>C (rs4149056)

Possession of three homozygous wild-type CYP2B6 15582C>T/ 516G>T/ 983T>C (CC/GG/TT) is predictive of EFV C_{24} in the lowest concentration stratum in a genome-wide association PK correlation study of ACTG trials. (Holzinger et al. 2012)

6.2.5 Study Enrolment

Screening procedures were common to both arms and both stages of the study, which were conducted in parallel; firstly, to group B investigating NANO-LPV, then to group A, the NANO-EFV cohort in the primary stages.

After analysis of the primary results by a protocol steering committee.

6.2.6 (NANO-EFV) GROUP A: PRIMARY STAGE PHARMACOKINETIC PROTOCOL

Three to five volunteers were to receive 50mg of NANO-EFV orally once daily over a 21-day period, with a witnessed dose on day 1 followed by a 72-hour PK profile (pre-dose within ten minutes of dosing, 2, 3, 4, 6, 8, 12, 18, 24, 36, 60 and 72 hours post-dose). Daily dosing from day 4-21 followed, with a second PK profile at steady state following the decay in plasma concentrations over 10 days (pre-dose within ten minutes of dosing, 2, 3, 4, 6, 8, 12, 18, 24, 36, 60, 72, 84, 132, 180 and 228 hours post last dose). In addition, during daily dosing a blood sample for PK trough analysis was taken on days 7, 14 and 17.

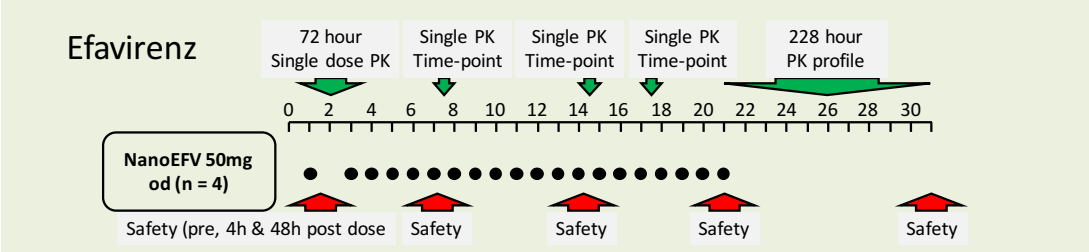


Figure 6:A Efavirenz protocol schedule of events

Efavirenz side effects related to the central nervous system were assessed by the volunteers completing a short 10-item questionnaire, grading symptoms on a four-point ordinal scale from none to severe.

6.2.7 (NANO-LPV) Group B Primary stage: Pharmacokinetic Protocol

Three to five volunteers were to receive 400mg of NANO-LPV orally twice daily over a 7-day period, with a witnessed dose on day 1 followed by a 12-hour PK profile (pre-dose within ten minutes of dosing, 1, 2, 4, 8, 12 hours post-dose). Twice daily dosing from day 1-7 followed, with a second PK profile at steady state following the decay in plasma concentrations over 3 days (pre-dose within ten minutes of dosing, 1, 2, 4, 8, 12, 24, 32, 48 and 56 hours post last dose). In addition, during twice-daily dosing a blood sample for PK trough analysis was taken on day 3.

From days 8 to 21 participants took no medication in a washout period.

On day 22, volunteers received 200mg of NANO-LPV twice daily with 100mg ritonavir (Norvir®) twice daily over a 7-day period, with a witnessed dose on day 22 followed by a 12-hour PK profile (pre-dose within ten minutes of dosing, 1, 2, 3, 4, 8, 12 hours post-dose). Twice daily dosing from day 22-28 followed, with a second PK profile at steady state following the decay in plasma concentrations over 3 days (pre-dose within ten minutes of dosing, 1, 2, 3, 4, 8, 10, 12, 24, 32, 48 and 56 hours post last dose). In addition, during twice-daily dosing a blood sample for PK trough analysis was taken on day 24.

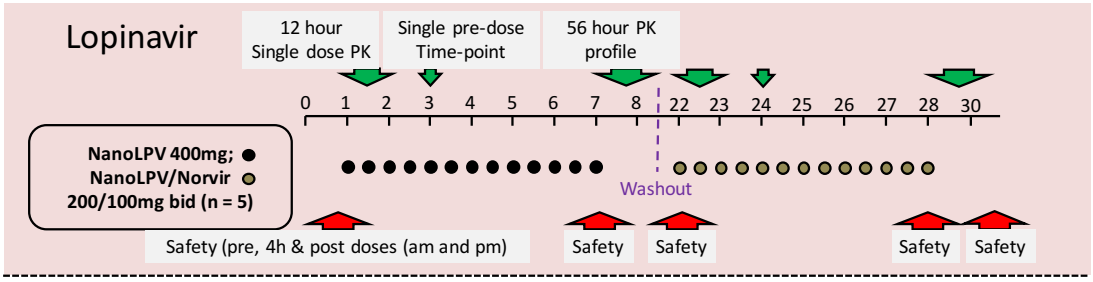


Figure 6:B Lopinavir protocol schedule of events

6.3 Primary Stage Results

At the time of this manuscript the SSAT055 remains an active ongoing clinical trial of investigational medicinal products. Both primary phase arms and the secondary phase NANO-LPV exploration have been completed, however the secondary phase efavirenz study has not yet been initiated.

Pharmacokinetic analyses and pharmacodynamic analyses have been conducted on samples from completed stages, however the formal GCLP compliant pharmacokinetic report is not yet available at the time of submission of this thesis. Similarly, data on individual participant's clinical history and safety had not undergone final reconciliation, quality checks and database lock, prior to clinical study report being compiled.

The results presented henceforth represent a compilation of the aggregated raw data and simple pharmacokinetic profiles.

6.3.1 Group A Primary Stage (NANO-EFV)

Four participants were enrolled to the primary stage of Group A and completed both single dose and steady state pharmacokinetic profiles having taken NANO-EFV 50mg once daily for 21 days.

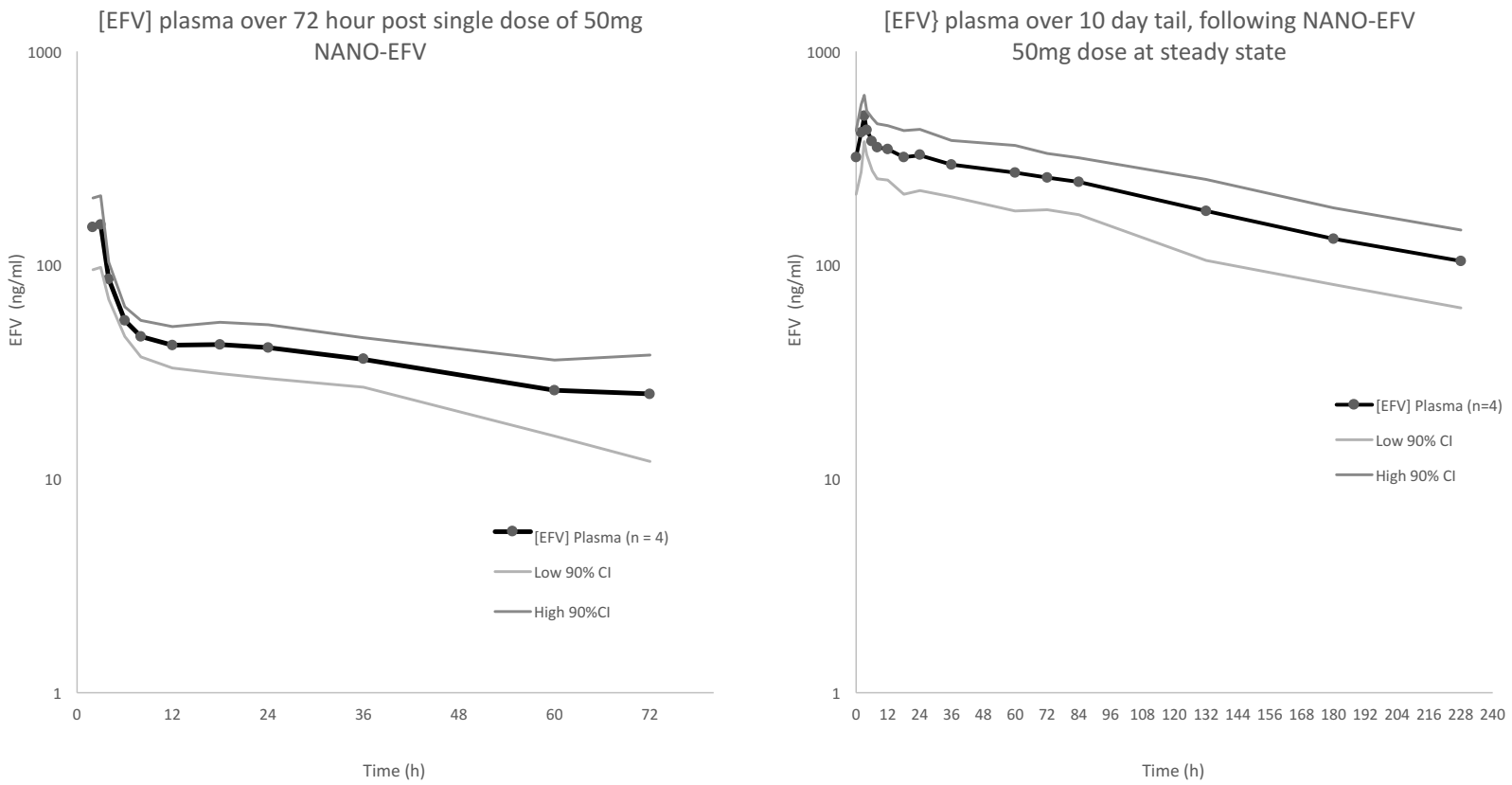
After the first dose: maximum plasma efavirenz concentrations C_{max} geometric mean (GM) with 90% confidence intervals was 155 ng/mL (98 – 211 ng/mL) measured at the 3 hour timepoint. At 24 hours, C_{24h} GM(90% CI) was 41ng/mL (29 – 52 ng/mL) and after 72 hours C_{72h} had declined to 25ng/mL, the limit of quantification.

At steady state, GM (90%CI) C_{max} efavirenz was again measured at 3 hours after dose at 501ng/mL (379 – 624 ng/mL) declining to 328 ng/mL (223 – 432 ng/mL) and 105 ng/mL (63 – 146 ng/mL) at C_{24h} and C_{228h}, respectively. Geometric mean PK profiles and 90% confidence intervals are illustrated in Figure 6:C

Table 6:B NANO-Efavirenz; single nucleotide polymorphisms in substudy participants

EFV patients (n=4)	Genotype frequencies		
CYP2B6 516G>T (rs3745274)	GG	GT	TT
	2	0	2
CYP2B6 983T>C (rs28399499)	TT	CT	CC
	4	0	0
***SLCO1B1* 521T>C (rs4149056)**	TT	CT	CC
	4	0	0

Single nucleotide genetic polymorphisms in CYP2B6 516G>T, CYP2B6 983T>C and SLC1B1 521T>C results are presented in Table 6:A but have not been analysed as covariates due to small numbers in the primary stage.



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2 *Figure 6:C Primary stage NANO-EFV, single dose and steady state PK profiles, Geometric mean (90% confidence intervals)*

6.3.2 GROUP B PRIMARY STAGE (NANO-LPV)

Five participants were enrolled to the primary stage of Group B and completed both 7 day dosing periods of 400mg NANO-LPV twice daily and after washout 200mg NANO-LPV + 100mg ritonavir twice daily, with 12 hour PK profile after single dose and 56-hour PK profile following the final dose on day 7 at steady state.

Geometric mean PK profiles and 90% confidence intervals for plasma lopinavir are displayed in Figure 6:D.

Single dose:

GM (90% CI) lopinavir concentrations peaked at 2 hours following a 400mg oral dose of NANO-LPV; C_{max} 111ng/mL (61-162) and were below LLQ in all participants at 8 hours post dose. Conversely after 200mg NANO-LPV with 100mg ritonavir, C_{max} occurred at 4 hours post dose; 976 ng/mL with high variability 90% CI (-1105 to 3057 ng/mL), declining to 645 ng/mL (-444 to 1734 ng/mL) at 12 hours post-dose.

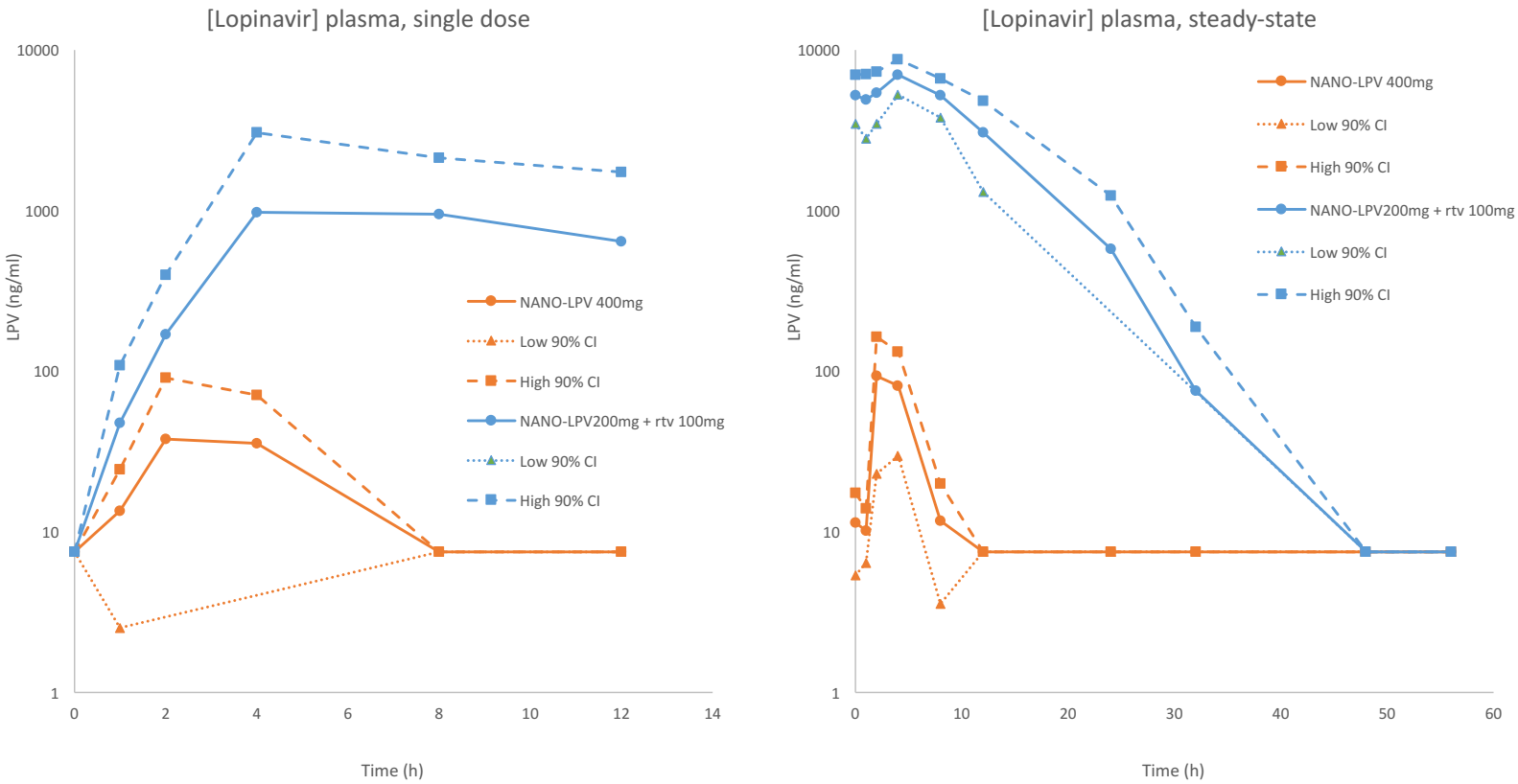
Steady State

After 7 days dosing with 400mg NANO-LPV twice daily, lopinavir C_{max} {GM, (90% CI)} was 93 ng/mL (23 – 164 ng/mL) at 2-hour post dose, with all below LLQ at 12 hours post dose.

Conversely, when NANO-LPV 200mg was taken twice daily with a dose of 100mg ritonavir, C_{max} was recorded 4 hours post dose at 7002 ng/mL (5263 – 8741 ng/mL), with C_{12h}, and C_{24h} were 3068 ng/mL (1307 – 4828 ng/mL) and 580 ng/mL (-84 to 1243 ng/mL), respectively and all concentrations were below the limits of quantification by 48 hour post-dose.

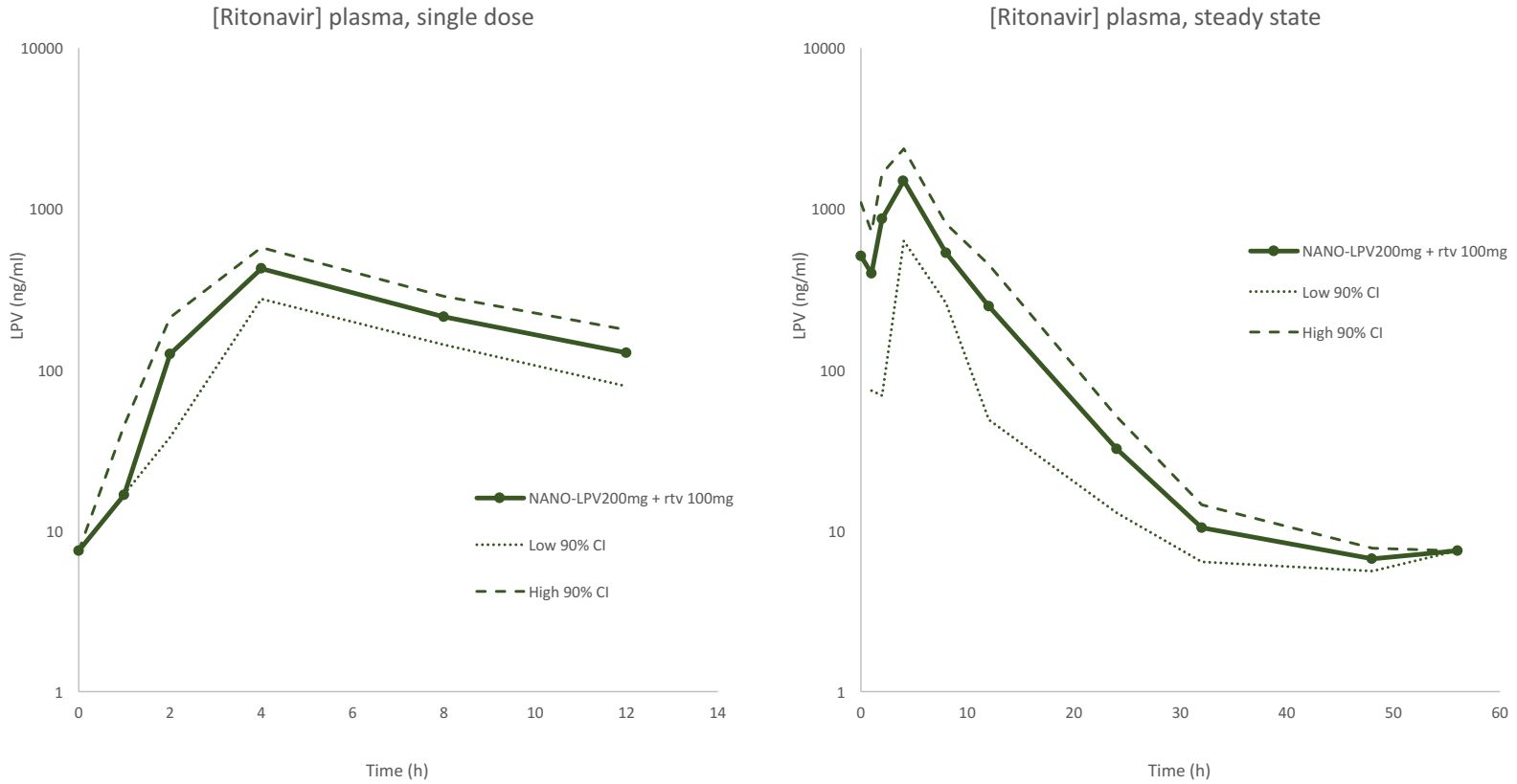
Ritonavir

Plasma ritonavir PK profiles after single dose and steady state in the ritonavir periods are shown in Figure 6:E.



1

2 *Figure 6:D Primary Stage Group B; Lopinavir plasma PK profiles with NANO-LPV +/- rtv at single dose and steady state, geometric*
3 *mean (90% confidence intervals)*



1

2 *Figure 6:E Primary Stage Group B; Ritonavir plasma PK profile when dosed with NANO-LPV at single dose and steady state, geometric*
3 *mean (90% confidence intervals)*

6.4 Modelling the Primary Stage Results to Inform Protocol Steering Committee

Nonlinear mixed-effects modeling was applied as described previously by Dickinson et al, (Dickinson et al. 2011) was developed to explore the relationship between lopinavir and ritonavir concentrations over 72 h after attaining steady state with NANO-LPV without or with ritonavir, in order to assess other lopinavir and ritonavir dosing strategies compared to the standard 400-mg–100-mg twice-daily dose.

The modelling methodology conducted by Dr. Dickinson is briefly described below.

Nonlinear mixed-effects modeling was applied by using NONMEM (version VI 2.0, level 1.1, double precision; ICON Development Solutions, Ellicott City, MD) (3) with first-order conditional estimation with interaction (FOCE-I). The model fit was assessed by statistical and graphical methods. The minimal objective function value (OFV) (equal to a 2-log like- lihood) was used as a goodness-of-fit diagnostic, with a decrease of 3.84 points corresponding to a statistically significant difference between nested models (P 0.05, 2 distribution, and 1 degree of freedom). Graphical diagnostics were performed with Microsoft Office Excel 2007 for Windows (Microsoft Corpora- tion, Redmond, WA). Standard errors of the parameter estimates were deter- mined with the COVARIANCE option of NONMEM, and individual Bayesian parameter and concentration estimates were determined with

the POSTHOC option. The model-building process was in 3 stages: (i) a separate model was developed for lopinavir, (ii) a separate model was developed for ritonavir, and, finally, (iii) a combined model was developed, incorporating the influence of ritonavir concentrations on lopinavir clearance

The Population PK method was used to describe the clinical data and to simulate alternative dosing strategies in order to inform PSC selection of

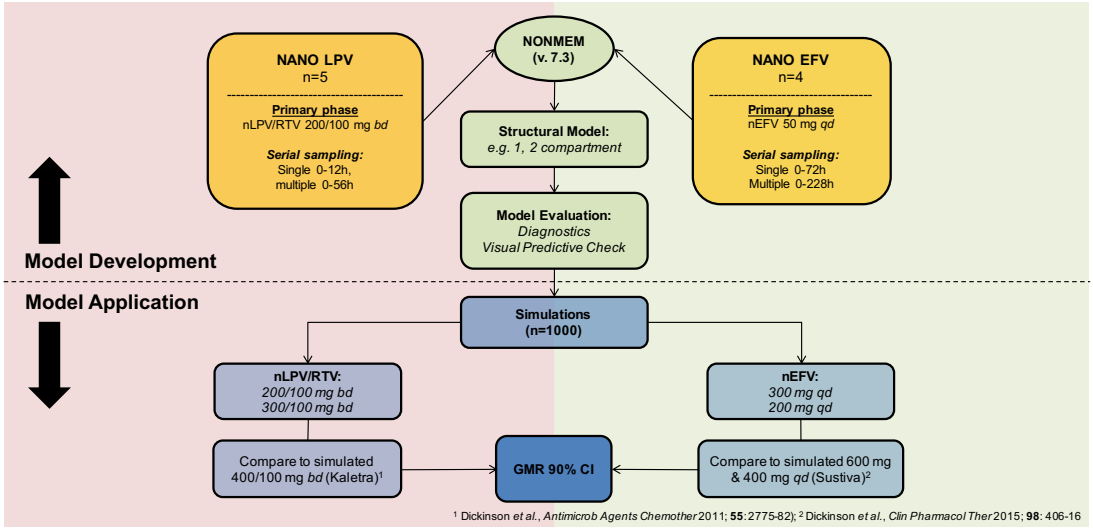
1. Kaletra® (LPV/RTV, 400/100 mg bid; n=16) LPV and RTV modelled using a sequential approach with a direct response E_{max} model (Dickinson et al. 2011)

2. NanoLPV/RTV (nLPV/RTV, 200/100 mg bid; n=5) nLPV and RTV modelled simultaneously with an indirect response E_{max}model (enzyme turnover; using only the multiple dose nLPV/RTV data)

Simulations:

1. LPV/RTV 400/100 mg bid, n=1000
2. nLPV/rtv 200/100 mg bid, n=1000
3. nLPV/rtv 300/100 mg bid, n=1000

Steady state was not assumed, but the model dosed with the same number and frequency of doses as the clinical study



A summary of the comparative simulation dosing with ritonavir boosted NANO-LPV 200/100mg compared to full-dose Kaletra and NANO-LPV/rtv 300/100mg is shown in Table 6:C. Comparing the 12-hour areas under the curve, 200/100mg NANO-LPV/rtv was predicted from the simulation to give comparable PK exposure to that of standard dose Kaletra, whilst the higher 300/100mg NANO-LPV/rtv

Table 6:C Population PK simulation output comparing dosing strategies, based on clinical data output from primary stage

Comparison of Kaletra *vs.* nLPV/RTV

Comparison of simulated C_{12}, C_{24}, AUC_{0-12}, AUC_{0-24}, and C_{max} made by means of GMR, 90% CI (difference considered significant if the CI does not cross 1 – shown in **bold**)

	Geometric mean			GMR (90% CI)*	GMR (90% CI)*
LOPINAVIR	***nLPV 200 mg***	***nLPV 300 mg***	***LPV 400 mg***	***nLPV 200 vs. LPV***	***nLPV 300 vs. LPV***
C_{12} (mg/L)	4.16	6.24	4.02	1.04 (0.99-1.08)	**1.55 (1.49-1.62)**
C_{24} (mg/L)	0.66	0.99	0.24	**2.77 (2.53-3.04)**	**4.16 (3.80-4.56)**
AUC_{0-12} (mg.h/L)	72.35	108.53	79.07	**0.92 (0.89-0.94)**	**1.37 (1.34 -1.41)**
AUC_{0-24} (mg.h/L)	101.64	152.46	100.76	1.01 (0.98-1.04)	**1.51 (1.47-1.56)**
C_{max} (mg/L)	10.69	16.04	9.97	**1.07 (1.05-1.10)**	**1.61 (1.57-1.65)**

	Geometric mean		GMR (90% CI)*
RITONAVIR	***RTV 100 mg (nano)***	***RTV 100 mg***	
C_{12} (mg/L)	0.214	0.123	**1.74 (1.57-1.92)**
C_{24} (mg/L)	0.017	0.013	**1.31 (1.06-1.61)**
AUC_{0-12} (mg.h/L)	9.46	3.59	**2.64 (2.54-2.74)**
AUC_{0-24} (mg.h/L)	11.85	4.33	**2.74 (2.62-2.88)**
C_{max} (mg/L)	2.08	0.59	**3.55 (3.43-3.67)**

* Nanoformulation as reference

NANO-EFAVIRENZ

Simulated doses of NANO-EFV 300 mg or 200 mg once daily were predicted approximate simulated doses of EFV (Sustiva) 600 or 400 mg once daily.

{Humanconfirmationo:2017wf}

6.4.1 Protocol Steering Committee

Both primary stages completed with no adverse events above grade 2, no discontinuation of therapy and no serious adverse events.

The Protocol Steering Committee consisted of the clinical investigators, members of the antiretroviral pharmacokinetic group and department of chemistry at the University of Liverpool, and an academic clinician and a biostatistician who were both extensively experienced in the clinical development studies of antiretroviral therapies. Guided by the results of the population PK simulation, the protocol steering committee agreed to proceed to the secondary stage investigation of NANO-LPV/rtv with the 200/100mg BD dose, comparing in two-phase randomised-order sequential dosing period with washout, against Kaletra® 400/100mg BD.

6.5 SECONDARY STAGE: NANO-LOPINAVIR

Ten volunteers were randomly allocated to Arm 1 or Arm 2 in a 1:1 allocation to determine the order in which they received the investigational product and nano-formulation.

The protocol for study conduct of the secondary stage was as described in section 6.2.7 above, with the minor alteration to add PK timepoints at 3 hour and 10 hour post-dose to better characterise the timing and magnitude of peak concentration.

Arm 1: Five volunteers received lopinavir/ritonavir as Kaletra® tablets at a dose of 400/100mg twice daily for 7 days, then following a 2-week washout period, received 200mg NANO-lopinavir and 100mg ritonavir (Norvir®) twice daily for 7 days.

Arm 2: Five volunteers received 200mg NANO-LPV and 100mg ritonavir (Norvir®) twice daily for 7 days, then following a 2-week washout period, received lopinavir/ritonavir as Kaletra® tablets at a dose of 400/100mg twice daily for 7 days

6.5.1 RESULTS

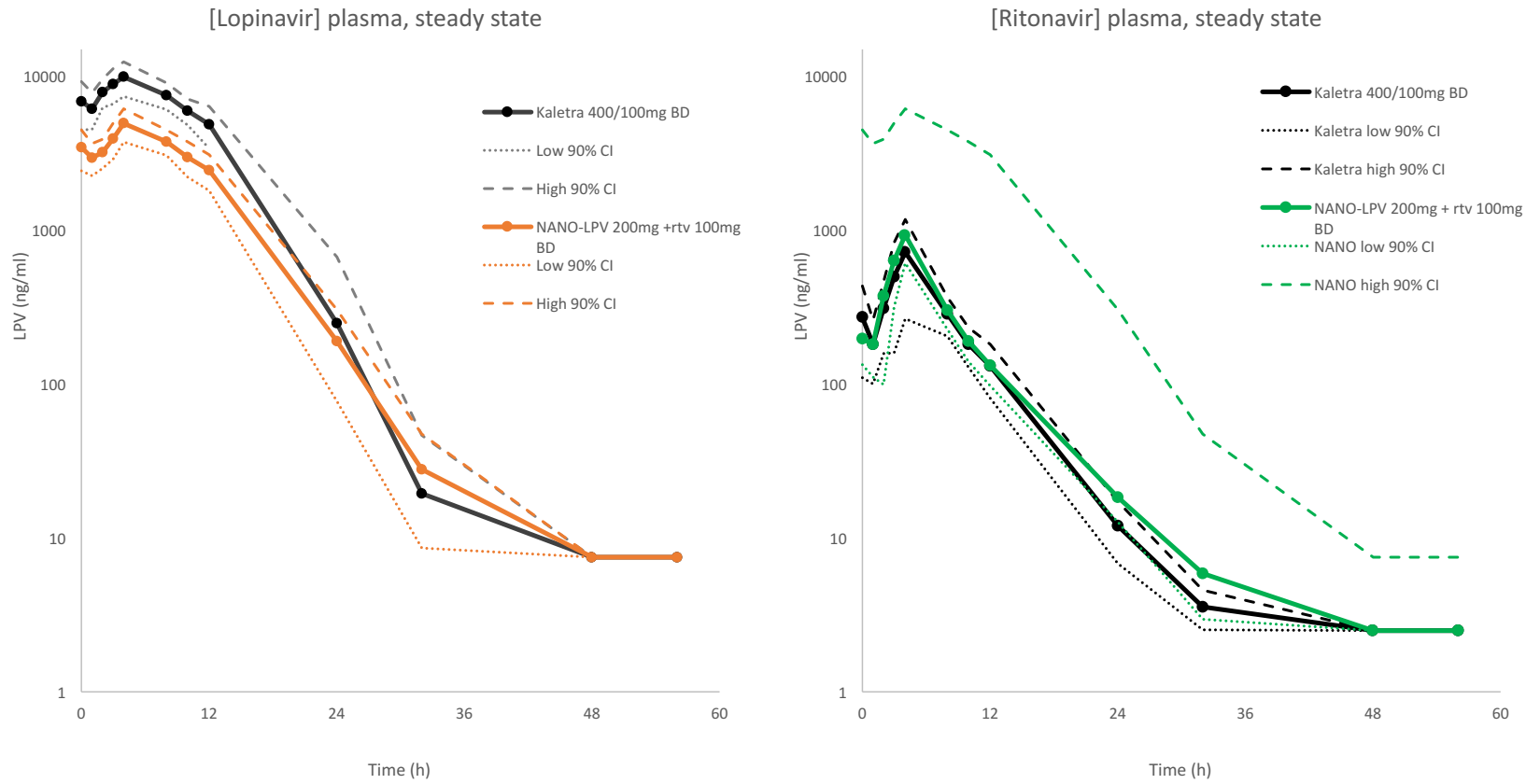
Steady State

After 7 days dosing with Kaletra® 400/100mg BD and NANO-LPV/rtv 200/100mg BD, steady state pharmacokinetic profiles for 56 hours post dose were obtained as plotted in Figure 6:F.

With Kaletra®, C_{max} GM (90% CI) of 9928 ng/mL (7418 – 12438 ng/mL) was attained at 4 hour post dose, with C_{12h} of 4880ng/mL (3392 – 6369ng/mL).

In comparison with NANO-LPV/rtv, C_{max} was 4954 ng/mL (3749 -6160 ng/mL) with C_{12h} at 2458 ng/mL (1807 – 3110 ng/mL).

Results of genomic DNA analysis for SNP of relevance to lopinavir metabolism for both stages are presented in Table 6:D. The predominant concordance between participants in genotype and small number of participants precludes further covariate analysis at this point.



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2 *Figure 6:F Secondary Stage: PK profiles (GM, 90% CI) for Kaletra 400/100 BD and NANO-LPV 200mg plus ritonavir 100mg BD, after*
3 *dosing to steady state for 7 days.*

Table 6:D NANO-Lopinavir pharmacogenomic results

LPV patients (n=13)	Genotype frequencies		
***CYP3A4**22 99366316G>A (rs35599367)**	GG	GA	AA
	13	0	0
***CYP3A5**3 6986A>G (rs776746)**	AA	AG	GG
	7	3	3
***SLCO1B1* 521T>C (rs4149056)**	TT	CT	CC
	12	1	0

6.6 Discussion

This preliminary data from an ongoing study pilot study, showed that both orally administered SDN formulations of efavirenz and lopinavir were generally well tolerated at the studied doses in small numbers of HIV-1 negative healthy volunteers. The data so far confirm the potential of a 50% dose reduction compared to the licensed formulations using a novel approach to the manufacture of solid dose nano-formulations.

These results are currently preliminary, and even on completion of this adaptive-design pilot study will have been limited to human studies in a less than two-dozen volunteers requiring further confirmation in larger studies. Regardless, the current European Medicines Agency guidance on investigation of bioequivalence (European Medicines Agency 2010) provides a good indication for a route to regulatory approval and how it could potentially be achieved at the next stage of human study.

The projected statement on the dose-reducing potential of the SDN for efavirenz compared to that of the licensed standard formulation is currently based on population PK modelling, using a common model developed within the same collaborative group to compare with a statistically-powered clinical study in HIV-1 infected patients receiving treatment; the ENCORE1 study. (Dickinson et al. 2016) This study established the non-inferior virologic efficacy of a 400mg dose of efavirenz compared to 600mg, both given with 2 NRTI. Though lower plasma PK exposures were observed with the 400mg dose, wherein the modelled predictions

for some participants indicated concentrations at 12 hours after dosing, C_{12h} below the putative minimum effective concentration of 1000 ng/L, no significant difference in the virologic failure rate was observed compared to those above this threshold.

On the basis of the ENCORE1 results, the 400mg dose of efavirenz is being considered by global non-governmental bodies for guidance approval with the intended benefit anticipated to be in low and middle-income countries with large populations infected by HIV. Ongoing studies are being conducted to provide evidence that this dose reduction does not have detrimental effects due to lowered therapeutic efficacy in two clinical situations of concern to healthcare in these societies; i) in women of child-bearing age during pregnancy and ii) when co-administered with rifampicin containing regimens for the treatment of tuberculosis. (Boffito et al. 2017)

With future confirmation of the bioequivalence of 200mg SDN formulation of efavirenz to standard formulation at 400mg, there are potentially significant savings to be gained both in cost of drug and in the amount of active pharmaceutical ingredient used within the context of a global manufacturing capacity which is not infinite (Table 6:E). If confirmed in larger future studies, the approach has the potential for savings up to 243 million USD per year while also freeing up manufacturing capacity up to 930 tons per year.

Table 6:E Predicted cost and product efficiency gains with the use of solid dose nanoformulations replacing standard formulations of lopnavir and efavirenz.

SDN (dose)	Comparator drug[1]	Est. cost of SDN pppy (USD)[2]	Est. savings in L&MICs during first 5 yrs of launch[3]	
			Cost saved (USD)	API saved
Lopinavir / ritonavir (200/100 QD)	400/100 BD.	~$ 183	~$ 216 Mn	331 tons
Efavirenz (300mg BD)	600 mg QD	~$ 24	~$ 471 Mn	~2,759 tons
	400 mg QD	~$ 24	~$ 113 Mn	~920 tons
Efavirenz (200mg BD)	400 mg QD (if approved)	~$ 16	~$ 314 Mn	~1,839 tons

1. Dose reduction predicted from the presented pharmacokinetic data
2. University of Liverpool estimated SDN process may add $4-16/kg to API cost, lower-end estimates shown here, updated with improved understanding of yield loss
3. High-level estimates based on ARV demand in low and middle income countries (L&MIC) 2015-2025 jointly forecast by Medicines Patent Pool & World Health Organisation (2016)

pppy = per person, per year

In a similar dose minimisation proposal to that described for standard formulation efavirenz (Dickinson et al. 2016), this group has previously investigated lopinavir (Jackson et al. 2011) and lamivudine (Else et al. 2012). All three could potentially be candidates for SDN formulation, as the approach has wide applicability to drugs from several classes for numerous indications. In addition to other development programmes for other oral applications, parenteral long-acting SDN applications are currently ongoing.

7 SUMMARY

In summarising the independent projects from which this thesis is constituted, each was designed with the aim of addressing a clinical question of relevance to current and future practice in the management of treatment and prevention of HIV. Each was successful in achieving its stated objectives, providing positive confirmations of the overriding hypothesis being tested.

A common feature of all three pieces of work was the incorporation as an integral feature of the design of the protocols of a feedback decision loop utilising pharmacokinetic methods to adjudge safety, efficacy and viability of the ongoing study. This is a common feature within pharmaceutical manufacturer's early stage human clinical studies with multi-phase single-ascending dose and multiple-ascending dose structures. In research from academic and healthcare research groups in the UK, this has previously been an uncommon strategy but is being used with increasing frequency with adaptive study designs. These often tend to be post-licensing studies which aim to explore and to define clinical effectiveness of the use of therapies, and may combine therapies to explore their synergistic effects. The potential for improving the efficient use of research funds, physical and human resources as well as the ability to build into safeguards based on target exposures within a therapeutic window, which has the ethical implications of avoiding exposure to futile doses, particularly in healthy volunteer studies. Within this context, the UK Health Research Authority has recognised the current and future trend towards the use of such study designs and this year has introduced specific

training on the research ethics pertaining to them, into the curriculum of the National Research Ethics Service training for committee members and chairs.

SSAT040 demonstrated that an nano-formulation of the non-nucleoside reverse transcriptase inhibitor, rilpivirine, when dosed intramuscularly was generally well tolerated as a single dose with no safety signals of concern apart from transient local injection site discomfort. Each studied dose provided prolonged plasma exposure for at least four weeks, at levels consistent with therapeutic efficacy. With regards its potential as a pre-exposure prophylaxis agent, the tissue drug penetration within the female genital tract and male rectum was comparable to plasma exposures. Testing these achieved female genital secretory fluid concentrations in an *ex vivo* model showed that viral inhibition could be achieved at these drug levels. On the basis of these study results the McGowan group at the Magee-Womens Research Institute in Pittsburgh, again supported by the same funder and manufacturer, have completed the next phase of study with 600mg and 1200mg doses in a protocol designed to specifically test viral inhibition in freshly biopsied rectal and vaginal tissue samples. (McGowan et al. 2016) Interestingly, though significant viral suppression was observed in rectal tissue, persisting for months after a single dose, no viral suppression was detected in cervical or vaginal tissue. This latter finding has had negative implications on the future development decision on long-acting rilpivirine for HIV prevention.

SSAT049 as a pilot study, demonstrated the efficacy of this oral once daily combination of darunavir, boosted by ritonavir with rilpivirine for effective viral suppression in small numbers of therapy naïve HIV-infected adults. At the time of creating the study protocol as a concept, the idea of co-formulating these agents to a single-tablet fixed dose combination was discussed in non-specific term with the drug manufacturer. Since that time, evolution of the therapeutic landscape has rendered these ideas moot, and in fact the manufacturer continued with the commercial decision to develop a co-formulation of darunavir boosted by cobicistat as a single-tablet, (Janssen-Cilag Ltd 2014) and very recently has received approval for both these agents combined with a dual-NRTI backbone of tenofovir alafenamide and emtricitabine in Symtuza®.(Janssen-Cilag Ltd 2017)

SSAT055, though not yet complete, has demonstrated the potential of a novel manufacturing process for solid-drug nanoparticle formulations and the potential for oral SDN formulations to provide advantageous pharmacokinetic exposure, with a reduction in the absolute amount of active pharmaceutical ingredient used. Whilst these advantages of dose reduction/ minimisation would be applicable to all global regions, the anticipated greatest benefit - should further development of the process chemistry and manufacturing be proven viable for large scale – is expected to be in low and middle-income countries where the majority of 19 million untreated people living with HIV reside. There has been a significant recent announcement to this regard from the World Health Organisation and the governments of South Africa and Kenya (with multiple global health stakeholders:

the Joint United Nations Programme on HIV/AIDS (UNAIDS), the Clinton Health Access Initiative (CHAI), the Bill & Melinda Gates Foundation (BMGF), Unitaid, the United Kingdom's Department for International Development (DFID), the United States President's Emergency Plan for AIDS Relief (PEPFAR), the U.S. Agency for International Development (USAID), and the Global Fund to Fight AIDS, Tuberculosis and Malaria, with Mylan Laboratories Limited and Aurobindo Pharma). A pricing and licensing agreement has been concluded to provide a generic, single tablet regimen containing the integrase inhibitor dolutegravir (with lamivudine and tenofovir disoproxil fumarate) to healthcare purchasers in low and middle-income countries for the price of US$75 per year {WorldHealthOrganisation:uy}. This is an important advance in the global access to antiretroviral therapies which addresses a major discrepancy between resource-poor and resource-rich countries; that of pricing and availability of guideline recommended first-line options (dolutegravir, efavirenz and nevirapine are the three WHO recommended third agent). The availability of a dolutegravir based single-tablet regimen at an accessible purchase price would appear to make efforts to optimise doses using SDN of the older third-agents, efavirenz and lopinavir/ritonavir, obsolete.

There are three counterpoints to this assumption which point to a continued demand for efavirenz based therapy. Firstly, the global prevalence of persons living with HIV are likely to continue to increase, due to a reduction in mortality with successful viral suppression whilst rate of decline in incidence lags behind. To achieve a ninety percent target of patients on therapy, will require significant additional capacity for manufacturing API for both existing products under license

agreements, as well as newly introduced products. Secondly, patients currently stable and established on efavirenz based regimens are likely to remain on their current therapy, thus maintaining this need for product and opportunity for its improvement. Finally, the proof-of-concept for oral SDN reformulations will likely apply in just the same way to other antiretroviral regimens.

8 ACKNOWLEDGEMENTS

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Firstly, I wish to thank my clinical supervisor at SSAT, Professor Marta Boffito, whose leadership, tutelage, guidance and encouragement were instrumental in stimulating an interest in clinical pharmacology and both initiating and completing this work.

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St. Stephen's AIDS Trust

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I wish to thank John Mellors, Kerri Jo Penrose, Urvi Parikh and Kristen Hamanishi who willingly made available their technical expertise, unique facility for virologic investigation and analysis which was critical to successful laboratory reconstruction of the events surrounding the seroconversion case which occurred.

Some of the results of this study were presented at the 19th Conference on Retroviruses and Opportunistic Infections, 5–8 March 2012, Seattle, WA. The study manuscript is published in Clinical Pharmacology and Therapeutics, June 2014. The

seroconversion case is published in the Journal of Infectious Diseases November 2015.

SSAT049 was supported by a grant from Janssen Pharmaceuticals. Preliminary results from this study were presented at the 21st Conference on Retroviruses and Opportunistic Infections, Boston MA, 3-6 March, 2014.

SSAT055 as a clinical study was supported by a funding grant held by Professors Owen and Rannard at the University of Liverpool. Portions of the results from this study were presented at the Conference on Retroviruses and Opportunistic Infections, Seattle WA February 13-16, 2017.

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